Pharmacokinetics, distribution, bioavailability and relationship to antibiotic residues

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PHARMACOKINETICS, DISTRIBUTION, BIOAVAILABILITY, AND RELATIONSHIP TO ANTIBIOTIC RESIDUES

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2.1 INTRODUCTION

To ensure public confidence, in relation to the consumption of foodstuffs derived from animals that have received antimicrobial drugs (AMDs), regulatory authorities adopt conservative approaches and set stringent standards on data requirements. Pharmacological, toxicological, and microbiological *no observable (adverse) effect levels* [NO(A)ELs] are determined and the lowest value is used to calculate the *acceptable daily intake* (ADI), which is the amount of drug or drug metabolite that can be consumed by humans daily throughout life without appreciable risk to health. The ADI is used to calculate the *maximum residue limits* (MRLs) (termed *tolerances* in the United States) of the selected marker residue, which is usually the parent drug but can also be a drug metabolite, the total concentration of several compounds, or a chemical conversion product of the parent compound plus metabolites. In support of MRLs in edible tissues of the target animal species, determined in residue depletion studies, companies seeking a marketing authorization (MA) for a product containing one or more AMDs are required to supply target species data on the pharmacokinetics and metabolism of the active constituents of the product, when administered at recommended dose rates. The pharmacokinetic studies provide quantitative data on the absorption, distribution, metabolism, and excretion of the drugs, involving in particular plasma or blood concentration–time profiles and identification and quantification of major metabolites.

This chapter summarizes the principal pharmacokinetic properties of the major groups of AMDs, particularly in relation to residues depletion. Regulatory control strategies are reviewed and for one jurisdiction, the EU, marker residues and target tissues are indicated for each group of compounds. The chapter also briefly reviews circumstances of therapeutic use, including prophylaxis, metaphylaxis, and therapy. The requirement for conducting residue studies on generic products for which bioequivalence to a pioneer product has been demonstrated is considered. Risk assessment, characterization, management, and communication, with respect to AMD residues in food derived from food-producing species, are summarized.

2.2 PRINCIPLES OF PHARMACOKINETICS

2.2.1 Pharmacokinetic Parameters

Pharmacokinetics is the science of describing quantitatively changes in drug concentration in the body over time as a function of administered dose. Generally, it is based on subjecting serum/plasma concentration–time data to mathematical models, which provide further data on absorption, distribution, metabolism, and excretion of the drug and its metabolites. Detailed discussion of the derivation, definition, and application of pharmacokinetic terms is outside the scope of this chapter (the reader is referred to reviews by Toutain and Bousquet-Mélou[1–4]). However, it is necessary to consider the plasma and tissue pharmacokinetics of AMDs in relation to residues in food-producing species. The relevant pharmacokinetic terms are defined in Table 2.1.

Chemical Analysis of Antibiotic Residues in Food, First Edition. Edited by Jian Wang, James D. MacNeil, and Jack F. Kay.

TABLE 2.1 Definition and Characterization of Pharmacokinetic Parameters

Term	Abbreviation	Dimension (Typical Units)	Estimation/Computation	Definition/Meaning
Area under curve	AUC	ATV^{-1} (μg h ml^{-1})	From raw data with trapezoidal rule or AUC = $F * \text{dose/Cl}$	Integral of plasma concentration–time curve; plasma (blood) exposure; internal dose is controlled by clearance and $F\%$
Maximum plasma concentration	C_{max}	AV^{-1} (μg ml^{-1})	Generally obtained from raw data; simple analytical solution for a monocompartmental model	Maximum plasma concentration after administration of a given dose
Time of maximum concentration	T_{max}	T(min, h)		Time of maximal plasma concentration (C_{max})
Clearance	Cl	VT^{-1} (ml/kg/min)	Cl = dose/AUC = $K_{10} * V_c$, where K_{10} is first-order rate constant of elimination from central compartment	Rate of drug elimination scaled by plasma concentration; expresses body's capacity to eliminate a drug; with F, the single determinant of plasma exposure
Volume of distribution in steady-state condition	V_{ss}	(Vl/kg)	V_{ss} = dose*AUMC/(AUC)^2, where AUMC is area under first moment of plasma concentration–time curve; V_{ss} = Cl*MRT, with MRT the mean residence time	Proportionality constant between amount of drug in body in steady-state condition and corresponding steady-state plasma concentration; term used to compute a loading dose
Volume of distribution; terminal phase	V_{area}	Vl/kg	V_{area} = Cl/terminal slope	Proportionality constant between amount of drug in body at a given time in terminal phase and corresponding plasma concentration; term used to compute a residual amount of drug from an observed plasma concentration located in terminal elimination phase
Terminal half-life	$T_{1/2}$	T(min, h)	Ln2/ terminal slope	Time required to halve plasma concentration during terminal phase; can be an elimination half-life when plasma decay is controlled by clearance and extent of drug distribution; can be a half-life of absorption when decay is controlled by rate of drug release from administration site (flip-flop situation)
Very late terminal half-life	$T_{1/2}$	T(h, day)	Ln2/ very late terminal slope	Time required to halve plasma concentration during a very terminal phase (called *gamma* (γ) phase in text for aminoglycosides); given a sufficiently sensitive analytical technique, it is possible to characterize a terminal half-life that has no clinical meaning but is relevant for residue depletion; this last phase may be viewed as a slow drug release from a deep but small compartment giving a situation analogous to a flip-flop from an injection site
Bioavailability	$F\%$	Scalar (percentage)	$F = (\text{AUC}_{EV}/\text{AUC}_{IV}) * 100$; when dose administered extravascularly (EV) is equal to dose administered intravenously (IV)	Express the amount of drug that is absorbed and gains access to central compartment after dosing by a nonvascular route

Notation: A = amount; V = volume; T = time.

When a drug is administered intravenously, that is, directly into the pharmacokinetic central compartment, there is no absorption phase and the plasma or blood concentration–time profile can be used to derive, by fitting regression lines to the data using appropriate computer programs (e.g., WinNonLin) three intrinsic properties of the substance: clearance (Cl), volume of distribution(s) (V), and elimination half-life (T_{max}). These are described as PK parameters and are generally obtained in healthy animals; when a collection of these PK parameters is obtained from a set of animals, they become statistical random variables with typical values, distributions (often lognormal), and other characteristics. The goal of so-called population kinetics is precisely to evaluate these statistical parameters and to explain variability (inter- and intra-animal) with different covariables, such as age, sex, and health status. This is relevant regarding the establishment of a withholding time (WhT) that needs to take into account all these factors of variability. For example, nothing guarantees that clearance of an AMD established in healthy animals is equal or even similar to clearance that could be obtained in diseased animals.

There are many more pharmacokinetic properties, such as C_{max}, T_{max}, area under the curve (AUC), absorption half-life, terminal half-life, and bioavailability, which are not unequivocally related to the drug substance but to a given formulation of the substance, namely, a drug product and for a given drug product, to the route of administration and circumstances of drug administration (e.g., whether administered to fed or fasted animals), and so on. From a residues perspective, this explains why a withholding time (WhT) cannot be a substance parameter but is a product characteristic depending on the route of administration, dosage regimen, and other parameters. In contrast, a MRL is a concentration that is a substance property, intrinsically independent of any kinetic characteristics of the substance, allowing authorities to fix its value as a "regulatory constant" having a universal meaning and that can be subjected to international harmonization.

The relationships between the different PK parameters and plasma drug concentrations (often reported as C_{max} and AUC) are indicated by the equations in Table 2.1. In simple terms, it will be seen that, for a given dose, if Cl_{tot} is high, AUC will be low, and this impacts on residues insofar as AUC (also termed *exposure* or *internal dose*) in plasma will relate to tissue concentrations (albeit in a possibly complex manner). Clearance is the genuine pharmacokinetic parameter expressing the body capacity to eliminate a substance and determining dose amount to administer to achieve a targeted plasma concentration (for a given bioavailability). The terminal half-life, which expresses a change in concentration in units of time, provides an easily understood parameter for describing a terminal concentration–time profile.

The terminal half-life is a hybrid parameter, and its determinants need to be acknowledged. It is either a hybrid PK parameter determined by both clearance and distribution of the substance or, when there is a flip-flop, a PK characteristic of the drug product depending on bioavailability factors (rate and extent of absorption). In this latter case, the terminal half-life does not reflect the intrinsic rate of substance elimination. Whatever the biological factors controlling the terminal half-life, $T_{1/2}$ should be considered to select an optimal interval between dose administrations. This is clear if one considers a threshold plasma concentration required for AMD efficacy; a long terminal half-life will result in a longer time for plasma concentrations to decline to the threshold concentration.

More importantly, in relation to residues of AMD, the existence or nonexistence of a so-called very late terminal phase needs to be considered. When using a sensitive analytical technique, a supplementary phase can be detected for a range of plasma concentrations that are below the microbiologically effective plasma concentration and thus without therapeutic meaning. This terminal phase decays very slowly (half-life typically higher than 24 h), and it reflects persistence of drug residues in some deep compartments. This terminal phase is actually controlled by the redistribution rate constant from tissue to plasma. Aminoglycosides provide an example of this situation with a therapeutically significant half-life of approximately 2 h, while a very late terminal phase decays with a much slower half-life (see Section 2.3.1), explaining the persistence of residues in some tissues for weeks or even months.

In addition, this very late terminal phase can lead to drug accumulation with repeated administrations, explaining that the WhT, required to fall below the MRL, can be much more prolonged after a multiple dosing regimen than after a single dose administration. It is only the remnant amount of drug during the very late terminal phase that is consistently higher after a repeated dosing regimen, while there is no therapeutically relevant accumulation in plasma concentration during the treatment itself (for further explanation, see Fig. 15 in Toutain and Bousquet-Mélou[4]).

In relation to residues of AMDs, the pharmacokinetic studies conducted in laboratory animals and target species required by regulatory authorities are designed to establish concentration–time profiles of parent drug and its biologically active and inactive metabolites in body fluids (usually blood, serum, or plasma). For laboratory animals, the purpose of comparative metabolism studies is to determine whether laboratory animals used in toxicological testing have been exposed to the metabolites that humans will be exposed to as residues in products of food animal origin.[5] For target species, metabolism studies are required to determine the nature and quantity of veterinary drugs residues.[6] This task is generally accomplished using radiolabeled drugs to cover all possible residues. Finally, target tissue

concentration–time profiles are determined after administering the recommended dosage schedule of the product in the formulation intended for clinical use in order to describe the marker residue depletion to establish product WhTs.[7] Ideally, pharmacokinetic data and metabolism studies are required for each target species, for each administration route, and both minimum and maximum dose rates, in the event of variable dosage recommendations. In the latter circumstance, residue depletion studies conducted with the highest recommended dose administered for the longest recommended duration are the minimum requirement.

2.2.2 Regulatory Guidelines on Dosage Selection for Efficacy

Guidelines on dose selection of AMDs vary between jurisdictions, but all require the sponsor to demonstrate in preclinical studies the pharmacokinetic profile in the target animal species and the pharmacodynamic profile against microorganisms. The latter comprises the spectrum of activity, whether the drug is primarily bacteriostatic or bactericidal (at clinically effective dose rates), and whether the type of killing action is primarily concentration-, time- or co-dependent. AMD pharmacodynamics may be quantified using several indices, the most important of which are minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), while growth inhibition–time curves are used to define both the type of killing action and the steepness of the concentration–effect relationship.

The most widely used hybrid indicator of both efficacy and potency is MIC. When MIC has been determined against a sufficient number of strains (usually hundreds because of the inter-strain variability in potency) of each sensitive microbial species, the median or geometric mean MIC_{50} and MIC_{90} values are determined. It is then possible to set a provisional dose through integration of pharmacodynamic and pharmacokinetic data, using one or more of the following indices: $C_{max} : MIC_{90}$ (for some concentration-dependent drug classes, e.g., aminoglycosides), $AUC : MIC_{90}$ (for most concentration- and co-dependent drugs, e.g., fluoroquinolones, macrolides, tetracyclines), and $T > MIC_{90}$ (for most β-lactam drugs). The latter is the proportion of the inter-dose interval for which plasma/serum concentration exceeds MIC_{90} and is expressed as a percentage of the inter-dose interval.

The scientific literature is replete with proposals for numerical values of these indices, for example, $C_{max} : MIC_{90} \geq 10:1$ for aminoglycosides, $AUC : MIC_{90}$ ratio $\geq$ 125 h for fluoroquinolones, and $T > MIC_{90} \geq 50\%$ for β-lactams. In fact, these values provide no more than a guide to clinically effective dosage for several reasons:

1. Target numerical values, in practice, are "bug and drug"-specific.
2. The dosage required is dependent on bacterial load and level of immune competence of the host animal.
3. The dosage required depends on whether the endpoint is clinical cure, bacteriological cure, or avoidance of emergence of resistance.

For further discussion of these indices, see Lees et al.[8–10] and Toutain et al.[11–13]

The approach to final dosage schedule determination, following provisional determination using PK-PD principles as described above, varies between jurisdictions and is not considered here in detail. One example is taken. In the EU, guidance is provided by European Medicines Agency/Committee for Veterinary Medicinal Products (EMA/CVMP, previously European Medicines Evaluation Agency/Committee for Veterinary Medicinal Products, or EMEA/CVMP), which recommends the conduct, using clinically relevant disease models in the target species, of dose titration/determination studies.[14] These should be conducted separately for each administration route, each proposed dose, and each disease indication; thus the requirements can be onerous in terms of both cost and animal welfare. The dose rate, which provides a greater response than the next-lower dose but no greater response (statistically) than the next-higher dose, is selected for further study in a dose confirmation study, which is conducted again in a disease model or in clinical subjects.

The problems/disadvantages of dose-ranging studies have been discussed elsewhere.[8,10,12,13] Briefly, the dose selected may be demonstrably *effective* by appropriate statistical analyses but is unlikely to be *optimal*. Lees et al.[8] and Toutain et al.[13] have recommended as an alternative the use of PK-PD modeling approaches (not to be confused with PK-PD integration), in which computer programs, utilizing, for example, the sigmoidal E_{max} equation, are applied to evaluate the whole sweep of the concentration–effect relationship. This enables determination, using *in vitro*, *ex vivo*, and *in vivo* techniques, of drug concentrations and dosages required to achieve specific levels of inhibition of bacterial growth and bacteriostatic or bactericidal eradication of bacteria.[15,16]

2.2.3 Residue Concentrations in Relation to Administered Dose

Residue concentrations and their depletion profiles are inevitably linked to administered dose of an antimicrobial drug, albeit in a possibly complex and tissue dependent manner. This is illustrated *first* by the equation linking, for a systemically administered drug, dose to area under the plasma/blood concentration–time curve:

$$\text{Dose} = \frac{\text{Cl} \times \text{AUC}}{F} \tag{2.1}$$

where Cl = whole-body clearance, AUC = area under plasma or blood concentration–time curve, and F = bioavailability, the proportion of the administered dose absorbed, and gaining access to the central compartment. Rearrangement of Equation (2.1) leads to the following equation:

$$\text{AUC} = \frac{\text{Dose} \times F}{\text{Cl}} \tag{2.2}$$

This equation illustrates that the higher the dose and value of F and the lower the value of Cl, the greater will be the amount (exposure) of drug in plasma/blood over a measured time interval. If, in a dose-ranging study, F and Cl are held constant (i.e., if the pharmacokinetics is linear), doubling the dose increases AUC by a factor of 2. The AUC will not increase in direct proportion to administered dose, however, if Cl and/or F are not PK parameters but rather dose-dependent variables, as is the case for non-linear pharmacokinetics. For example, F may be lower at higher dosages of orally administered drugs that are highly lipid-soluble but poorly water-soluble, while Cl may be slower at high doses as a consequence of saturation of elimination pathways.

Secondly there will be a relationship between drug concentration in plasma (the driving concentration) and concentration in tissues, but the two will rarely be equal, and tissue concentrations will depend on a range of drug properties (see Section 2.2.6) and animal characteristics. It is important to note the differences in importance and consequence of total tissue concentration for pharmacological/toxicological responses on one hand and for drug and metabolite residues on the other hand. For the latter, it is the mean tissue concentration (not the separate concentrations in extracellular or intracellular fluids or intracellular distribution between several compartments) that determines intake by human consumers. In contrast, for the former, tissue concentration has limited (if any) value and may actually be misleading. This is illustrated by the macrolide, lincosamide, and pleuromutilin groups of antimicrobial drugs. Drugs of these classes, in magnitudes varying from compound to compound, achieve high overall concentrations in lung tissue, but the highest concentrations occur at intracellular sites. This circumstance is of no benefit therapeutically if the biophase where organisms are located is an extracellular site (such as epithelial lining fluid), as is the case for most bacterial species causing lung infections in farm animal species. This circumstance may be likened to an army confined to barracks and unable to contribute to the fight waging on the battlefield outside.

In relation to drug residues, a pharmacokinetic property of major significance is the very late terminal elimination half-life (see Sections 2.3.1 and 2.7.1). For many drug classes, a semi-logarithmic plot of plasma concentration versus time, after intravenous dosing, reveals a multicompartmental model, with three phases describing the compartments, of slopes λ_1, λ_2, and λ_3. These represent, respectively, rapid distribution, slow distribution, and finally, after reaching a pseudoequilibrium distribution (i.e., when the same amount of drug is exchanged from central to peripheral compartments and vice versa, from peripheral to central compartments) the decay observed during the terminal phase corresponds to the net elimination process. The third phase may be revealed only if (1) sampling is continued beyond concentrations of therapeutic relevance and (2) the analytical method is sufficiently sensitive (i.e., has a low lower limit of quantification). For most drugs the λ_2 phase is of therapeutic interest, as it determines the interval between doses required to provide clinical efficacy. On the other hand, the λ_3 phase (also named *gamma phase* in the literature) represents for some drugs the slow decline in concentration of drug beyond the therapeutically useful concentration, as drug is off-loaded from tissues. The λ_3 phase represents drug elimination from what are termed *pharmacokinetic deep compartments*. Alternatively, the λ_3 phase may represent flip-flop pharmacokinetics for a fraction of the drug that is slowly absorbed (see Sections 2.3.1 and 2.7.1). In both instances, it is the λ_3 phase value that normally determines WhTs.

There are two equations which can be used to represent terminal half-life:

$$T_{1/2} = \frac{\ln 2}{\text{Terminal} \times \text{slope}} = \frac{0.693}{\lambda_3} \tag{2.3}$$

This equation is the mathematical expression of the definition of a half-life, specifically, the time required for plasma concentrations to be divided by 2 after reaching pseudoequilibrium; as λ_3 is a hybrid parameter related to V_{area} (the volume of distribution associated with the terminal phase) and plasma clearance, Equation (2.3) can be re-written in a more mechanistically useful way as follows:

$$T_{1/2} = \frac{0.693 \times V_{\text{area}}}{\text{Cl}} \tag{2.4}$$

Equation (2.3) is conceptually useful in indicating that, when slope (λ_3) is shallow, half-life will be long and therefore WhT will also be long. Equation (2.4) is mechanistically useful in illustrating that $T_{1/2}$ depends on Cl, rate of elimination from the body, and V_{area}, on the extent of distribution within the body. Clearly, if Cl is slow, $T_{1/2}$ will be prolonged.

Volume V_{area} is a proportionality constant, indicating the relationship between a plasma concentration in the terminal phase and the corresponding total amount of drug in the body. It may be useful to perform such a computation to compare, at a given time, the total residual amount of drug in the body and the ADI. It should be stressed that V_{area} does

not represent a particular physiological space, and if one wishes to discuss physiological drug repartition and WhT, the steady-state volume of distribution (V_{ss}) is the most appropriate volume of distribution to be considered because it is physiologically based and its numerical value (always lower than that of V_{area}) directly indicates equilibrium distribution mechanism, whereas V_{area} is also influenced by plasma clearance. However, interpretation of a V_{ss} to anticipate a WhT is not straightforward as a high value of V_{ss} (e.g., much greater than body water volume) may represent uptake in high concentration into intracellular fluid of most or all body cells. Alternatively, it may represent uneven distribution and a high concentration in a specific tissue or tissues. Some drugs can have prolonged WhTs because of association to a large V_{ss}, but a low V_{ss} does *not* guarantee a short WhT (see Section 2.3.1), as a drug may achieve high concentrations in one tissue, such as kidney (e.g., aminoglycosides), while its *overall distribution* is limited.

The many factors that can alter V_{ss} and/or Cl, and thereby shorten or prolong $T_{1/2}$ are discussed in detail elsewhere.[1–4,17] They include altered fluid balance, nutritional status, percentage of body fat, species, hormonal status, age of animal, and disease status. For example, renal and/or hepatic disease can reduce Cl and therefore prolong $T_{1/2}$ for the therapeutic phase, while infectious diseases may either increase or decrease Cl and/or V_{ss}. In contrast, the main factor controlling the slope of the very late terminal phase is the redistribution of the drug from a deep compartment to plasma.

2.2.4 Dosage and Residue Concentrations in Relation to Target Clinical Populations

The efficacy and safety of antimicrobial drugs depend on both pharmacodynamic (drug efficacy and potency against the disease causing organism) and pharmacokinetic (exposure of organisms in the biophase for sufficient time to provide bacteriological cure) properties. Both properties must therefore be used in selecting a rational dosage schedule for clinical use. Regulatory authorities, therefore, require pharmaceutical companies to supply pharmacokinetic data in healthy and homogeneous animals (i.e., animals selected to minimize sources of interanimal variability). The requirements are different for residue studies where regulatory authorities explicitly require that animals used to identify the nature of residues be representative of the target population.[7] For example, if there are reasons to believe that the metabolisms of non-ruminating cattle will significantly differ from those of adult cattle, two separate studies will be required to document the possible influence of age on drug metabolism and on the nature of residues.

Similarly, separate studies are recommended when the target population includes both pre-ruminant and ruminant cattle to establish product WhT. However, the health status is ignored; this is of concern especially for AMDs. According to Nouws, the disease state is the main factor affecting the WhT.[18] This author determined tissue residue concentrations and persistence of different AMDs, including β-lactams, aminoglycosides, tetracyclines, macrolides, chloramphenicol, and sulfonamides in normal and emergency-slaughtered ruminants after parenteral or intramammary administration. At that time analytical assays (microbiological assays) were rather crude and MRLs were not established. Nevertheless, it is interesting to note that, comparing with the same pharmacokinetic model normal and emergency-slaughtered cattle, Nouws concluded that to predict WhT for muscle and kidney it was necessary to multiply by a factor of 2–3 or 4–5, respectively, values obtained in normal cattle to predict values in emergency-slaughtered ruminants.[19] No recent studies using current analytical methods have been performed to update these data, but it is very likely that residue depletion of AMDs is not equivalent in healthy and diseased animals.

A solution to this difficulty would be to define the depletion profile in clinical subjects or in disease models that closely simulate clinical disease. No attempt is made to meet this ideal for a range of ethical, economic, and scientific reasons. Instead, reliance is placed on conducting residue (like pharmacokinetic) studies in healthy animals. This is justified by the series of conservative assumptions made in establishing withholding periods (see Section 2.5.4.1).

Another important topic for regulatory authorities concerns flexibility in selecting dose schedules for clinical use for a given claim in a given species. We have argued elsewhere for greater reliance on (1) PK-PD modeling approaches[8] and (2) population pharmacokinetic studies as alternatives to classical dose titration studies in disease models[20] in order to optimize dosage for clinical and bacteriological cures.[13] However, a tailored dosage regimen taking into account both PK and PD variability raises the question of the WhT that has a single value. An advance would involve proposing a range of dosage regimens and establishing corresponding lower and upper bounds for the WhT.

2.2.5 Single-Animal versus Herd Treatment and Establishment of Withholding Time (WhT)

In poultry and porcine husbandry in particular, the use of AMDs "in feed" or "in drinking water" for prophylaxis, metaphylaxis (or control in the United States) or therapy is widely practiced (see Section 2.7.3.2). Prophylaxis involves administration of AMDs to healthy animals known to be at risk (as a consequence, e.g., of close proximity of animals housed together or predictable stresses caused by transport or adverse weather conditions). Metaphylaxis involves

administration of AMDs, again to animals judged to be clinically healthy, but which are in contact with animals in which clinical signs have been detected. With such group dosing procedures, the dose received by individual animals is likely to vary considerably. This is in part a simple consequence of provision to the group of medicated feed or water, but variable intake may be compounded by smaller animals losing out in competition for access to feed or water. Even worse is the disinclination of more severely diseased animals in the group to eat or drink the medicated food or water. Moreover, drug intake is discontinuous in each animal. From a residue perspective, these sources of variability in drug intake inevitably have direct consequences for variability in tissue residues, and this should be considered when WhT is established in an experimental setting where administered doses are carefully controlled.

In contrast, the therapeutic use of AMDs generally involves treating animals individually with AMDs formulated for parenteral (usually intramuscular or subcutaneous) or oral dosing, with animals dosed on a mg/kg body weight basis. Here, dosing can be more accurate even if body weight is normally assessed rather than measured under clinical conditions.

2.2.6 Influence of Antimicrobial Drug (AMD) Physicochemical Properties on Residues and WhT

With long and expensive drug development times, there is a need in the pharmaceutical industry to optimize the drug discovery process. For human drugs, "Lipinski's rule of 5" aims at predicting oral drugability of new drug candidates by computing or measuring a set of descriptors, including the substance molecular weight, octanol–water partition (expressed as log P) to assess lipophilicity/hydrophilicity, number of hydrogen-bound acceptors or donors, and so on. Considering some cutoff values (actually 5), it can be predicted if the substance is likely to have desirable pharmacokinetic properties. For veterinary drugs, the question of residues and WhT is a critical factor that should be documented very early in the development program. However, currently no systematic investigation is carried out to link residue persistence in tissues and physicochemical properties of the active ingredients, thus allowing development of a general rule comparable to the Lipinski rule. To succeed in this objective, it would be necessary to investigate the residue depletion curve after intravenous administration to establish the contribution of the substance itself versus all other factors (mainly formulation) on the WhT. Currently, it is recognized that tissue concentrations will depend on a range of properties of the drug, namely, lipid solubility, acidic/basic characteristics, which influence the passive diffuse of drug across cell membranes and, for a few drugs, active uptake by or extrusion from tissues.

A low, moderate, or high degree of lipid solubility can have profound effects on AMD pharmacokinetics and tissue residues. Table 2.2 presents a broad classification of drugs on the basis of lipid solubilities and summarizes the impact on pharmacokinetic profiles. Drugs of high lipid solubility are organic molecules, which are un-ionized or only partially ionized at physiological pH. AMDs of low lipid solubility are usually either strong acids (e.g., penicillins) or strong bases (e.g., polymyxins) and hence wholly ionized at physiological pHs. Aminoglycosides are weak bases but nevertheless highly polar and very poorly lipid-soluble, due to the presence of sugar residues in the molecules.

As drug residue science is concerned with metabolites, as well as parent molecules, it should be noted that most (especially phase II) metabolites are more polar, less lipid-soluble, and less biologically active than parent drugs. Hence, most metabolites follow the general rules on disposition (poor penetration of cell membranes) and elimination (high concentrations in urine and/or bile) applicable to poorly lipid-soluble drugs.

2.3 ADMINISTRATION, DISTRIBUTION, AND METABOLISM OF DRUG CLASSES

2.3.1 Aminoglycosides and Aminocyclitols

The principal drugs of the aminoglycoside class are streptomycin (which is not extensively used in veterinary medicine because it is less safe than dihydrostreptomycin), dihydrostreptomycin, gentamicin, amikacin, kanamycin, apramycin, tobramycin, neomycin, and paromomycin. Aminoglycosides characteristically comprise an aglycone linked to one or more sugar units (a glycosamine and/or a disaccharide). In the aminocyclitols (e.g., spectinomycin), the amino group occurs in the cyclitol ring. The pharmacokinetics of aminoglycosides are dictated by their highly polar and poorly lipid-soluble physicochemical properties; these respective solubilities in water and lipid are related to both their polycationic nature and the fact that they contain "sugar" residues, such as streptose in streptomycin and dihydrostreptomycin.

Absorption extent from the gastrointestinal tract (GIT) is very low (of the order of $\leq$1–2% of administered dose), although higher bioavailability may be achieved in neonatal animals and where there is disruption of the intestinal mucosa, caused by, for example, parvovirus infection. Within the GIT, aminoglycosides are stable and excreted unchanged in feces.

When AMDs are administered parenterally as aqueous solutions (usually intramuscularly), absorption into the

TABLE 2.2 Influence of Lipid Solubility of Antimicrobial Drugs on Pharmacokinetic Properties (ADME)[a]

Drugs with Low Lipophilicity		Drugs with Moderate to High Lipophilicity			
Strong Acids	Strong Bases or Polar Bases[b]	Weak Acids	Weak Bases	Amphoteric	Drugs with High Lipophilicity
Cephalosporins, penicillins	Aminocyclitols, aminoglycosides, polymixins	Sulfonamides	Diaminopyrimidines	Most tetracyclines Chlortetracycline Oxytetracyline	Lipophilic tetracyclines Doxycycline Minocycline Fluoroquinolones Ketolides Lincosamides Macrolides Phenicols Rifamycins Triamilides
Penetrate cell membranes poorly or not at all; limited or no significant absorption from GIT except for acid-stable aminopenicillins, which have moderate but species-variable absorption; distribution limited mainly to extracellular fluids; concentrations in intracellular fluid, CSF, milk, and ocular fluids low, but effective concentrations may be reached in synovial, peritoneal, and pleural fluids; some penicillins actively transported out of CSF into plasma; generally excreted, usually in urine, in high concentrations as the parent molecule; some drugs actively secreted into urine and/or bile; biotransformation (e.g., in the liver) usually slight or absent		Readily cross cell membranes; generally moderate to good absorption from GIT but species-dependent; effective concentrations achieved in intra- and transcellular as well as extracellular fluids except for poor penetration of sulfonamides into intracellular fluid due to acidic environment; ability to penetrate into CSF and ocular fluids depends on plasma protein binding (e.g., most sulfonamides and diaminopyrimidines penetrate well); weak acids are ion-trapped in fluids alkaline relative to plasma, such as herbivore urine; weak bases are ion-trapped in fluids acidic relative to plasma (e.g., prostatic fluid, milk, intracellular fluid, carnivore urine); commonly dependent on biotransformation for termination of activity but may also be excreted unchanged in urine and/or bile; some drugs actively secreted into bile			Cross cell membranes very readily; generally well absorbed from GIT in monogastric species; penetrate into intracellular and transcellular fluids (e.g., synovial and prostatic fluids and bronchial secretions); also penetrate well into CSF, except tetracyclines and rifampin; termination of activity dependent on a high proportion of administered dose being metabolized, for example, in the liver but also at other sites (e.g., kidney, enterocytes); some drugs actively secreted into bile

[a] Absorption, distribution, metabolism, and excretion.
[b] Polymixins are strong bases, while aminoglycosides and aminocyclitols are weak bases, but polar and poorly lipid-soluble because of the presence of sugar residues in the molecules.

systemic circulation is rapid. Maximum concentrations in plasma are achieved within 14–120 min.[21] Plasma protein binding is low (<20%), but distribution is limited largely to extracellular fluids (plasma+interstitial fluid). Aminoglycosides penetrate poorly into cells, transcellular fluids, and milk, but urine concentrations are high, as reabsorption by passive diffusion into the systemic circulation is very limited. However, they bind to brushborder vesicles and cell membrane phospholipids in cells of the proximal convoluted tubule, probably through their free amino groups. The consequences are twofold: (1) they are nephrotoxic, and this is most significant for the most ionized compounds (e.g., neomycin), which exhibit the greatest binding affinity; and (2) binding is firm, with little or no reabsorption into peritubular capillaries. Hence, concentrations in the kidney cortex in excess of MRL persist for months rather than weeks. As enzymes capable of metabolizing AMDs are located intracellularly in the liver, kidney, and enterocytes, aminoglycosides are excreted almost entirely unchanged.

Papich and Riviere report marked variability in aminoglycoside pharmacokinetics (distribution, clearance, and half-life) with altered physiologic or pathologic states, including pregnancy, obesity, dehydration, immaturity, sepsis, endotoxemia, and renal disease.[21] The latter influence is predictable from the fact that body clearance is dependent almost entirely on renal excretion. Martin-Jimenez and Riviere concluded that aminoglycoside pharmacokinetics can be predicted across species by population pharmacokinetic modeling.[22]

The volume of distribution of aminoglycosides is increased in young calves relative to adults, as a consequence of high extracellular water volume relative to body weight, because volume of distribution is proportional to plasma volume. Volumes of distribution are lower in obese animals, as aminoglycosides penetrate very poorly into adipose tissue. Overall, volume of distribution ($V_{d,area}$) is of the order of 0.15–0.45 l/kg, and the clinically relevant terminal half-life (β phase) is 1.0–2.0 h.

For some aminoglycosides, it is necessary to note, from both pharmacokinetic and residue perspectives, that they comprise a mixture of compounds. Gentamicin, for example, is a mixture of four compounds, C_1, C_{1a}, C_2, and C_{2a}; residues are usually determined as the sum of these compounds. For neomycin, the residues are determined as neomycin B and for kanamycin, as kanamycin A.

The disposition of aminoglycosides is generally best described by a three-compartment model; the α, β, and γ phases represent, respectively, distribution half-life, the clinically relevant decay phase (which dictates dose schedules for therapy), and a final slow-release elimination of drug sequestered in tissues, particularly renal cortex and liver. The γ (classified above λ_3) phase determines residue depletion profiles. The α phase is rapid (≤60 min), the β phase is also generally short (≤5 h), but the γ phase is much longer, in farm animal species ranging for gentamicin from 11.0 h in the pig, 44.9 h in cattle, to 142 h in the horse.[21] Most of the administered dose is actually eliminated during the short β (classified above λ_2) phase that correlates well with the glomerular filtration rate (GFR) as there is virtually no reabsorption and no tubular secretion in the mammalian nephron. As GFR does *not* increase in proportion to body weight, elimination half-life tends to be longer in larger animals, and clearance decreases as body weight increases.[22] The latter authors also demonstrated dependence of the slow γ phase on dose and administration route, with considerable differences also in findings from different laboratories for a given species.

The prolonged persistence of aminoglycosides in renal cortical tissue increases the possibility of non-compliant tissue residues. This problem is compounded by the inter-animal variability in depletion from renal tissue, so that an original proposed WhT for gentamicin of 18 months for adult cattle was followed by a proposal to avoid usage altogether. A WhT of 40 days has been proposed for neonatal piglets.[21] As well as in the kidney, high concentrations of aminoglycosides occur in the liver.[23,24]

Gentamicin is the most frequently studied aminoglycoside and may be taken as an example for the group. Reported bioavailability after intramuscular dosing is 93% in cattle, 87% in horses, and 60% in catfish, and is similar for different muscle sites. It is also similar, but with slower absorption, after subcutaneous dosing, while oral bioavailability is virtually 0%. Oukessou and Toutain reported lower clearance and volume of distribution and higher plasma AUC values in sheep administered a low-protein diet, compared to those receiving a high-protein diet.[25] Pharmacokinetic parameters and variables for a wide range of species and doses are given by Papich and Riviere.[21] Although 90% of administered drug is recovered from urine within 24 h in calves and adult cattle, drug residues nevertheless persist in renal cortical tissue. On the other hand, drug concentrations in skeletal muscle are low. Other aminoglycosides used in farm animal medicine are apramycin, used in feed for treatment of porcine colibacillosis, and dihydrostreptomycin, which is used in combination with penicillins, notably procaine benzylpenicillin, in parenteral formulations.

Spectinomycin is an aminocyclitol with physicochemical properties similar to those of the aminoglycosides; it is polar and poorly lipid-soluble, but does not contain aminosugars or glycosidic bonds. Unlike aminoglycosides, it is not renotoxic. Oral bioavailability is low (<10%), and volume of distribution is small. The terminal half-life in cattle is short (1.2–2.0 h) after intravenous and intramuscular dosing. It is used mainly in pigs and poultry as a powder for solution in drinking water or as a feed additive, and in cattle, poultry, and pigs by intramuscular injection.

2.3.2 β-Lactams: Penicillins and Cephalosporins

The presence of a carboxylic acid grouping confers on all β-lactams a moderate to strongly acidic character. Benzylpenicillin, for example ($pK_a = 2.7$), is for all practical purposes wholly ionized at the pH of all body fluids, with the exception of gastric juice in monogastric species. The ionised: un-ionised molecule ratio exceeds 50,000 : 1 at a blood pH of 7.4. Therefore, most β-lactams do not readily cross cell membranes, so that intra- and transcellular fluid concentrations are low relative to plasma. Absorption from the GIT varies between drugs, with bioavailability of the order of 1–2% for benzylpenicillin, which is unstable in aqueous solution, especially at extremes of pH (e.g., in gastric juice). Improved bioavailability after oral dosing occurs for phenoxymethyl penicillin, ampicillin, and amoxicillin, in ascending order. This relates to the greater stability of aminopenicillins in acid media. The aminopenicillins also contain a basic amino group and are therefore amphoteric. There is also some likely species variability in oral bioavailability with low values (5–10%) quoted for amoxicillin in the horse, compared to 64–77% in the dog and 23% in the pig.[26] Absorption is improved in species such as the horse in products containing esters such as pivampicillin, but these compounds are not used in food-producing species. Aminopenicillins are poorly absorbed in pre-ruminant calves, and bioavailability is even less in ruminating calves. Many cephalosporins also have

moderate to good bioavailability after oral administration in monogastric species.

When β-lactams are formulated as aqueous solutions for parenteral (intramuscular or subcutaneous) use, they are rapidly absorbed to achieve maximum concentrations in 0.25–1 h. In many cases, therefore, the plasma concentration–time profile is very similar for intravenous and intramuscular routes.

The distribution and elimination of β-lactams is determined largely by their polar and generally non-lipophilic nature. Although individual drugs may be exceptional, the general rule is that β-lactams do not readily penetrate to intracellular sites of enzymes capable of metabolizing AMDs, so that they are excreted mainly as parent drug. Limited metabolism involves opening of the unstable, four-membered β-lactam ring to form, for example, amoxicilloic acid from amoxicillin. In the kidney the unbound fraction of parent drug is filtered at the glomerulus and excreted in urine with minimal absorption, so that by this mechanism alone the urine : plasma concentration ratio may be of the order of 100 : 1 (for free plasma drug concentration). However, many penicillins and cephalosporins are substrates for transporters in the proximal convoluted tubule, which promote the active secretion of specific organic acids from the peritubular capillaries into tubular lumen fluid. Therefore, from the combined effect of glomerular ultrafiltration and tubular secretion, the urine : plasma concentration ratios can be as high as 400 : 1.

For most penicillins in most species, binding to plasma protein ranges from low (30%) to moderate (60%). The clearance of most penicillins (non-protein-bound fraction) thus exceeds GFR and confers on most drugs rapid clearance and short terminal half-lives, of the order of 0.6–2.0 h, regardless of species. In addition, volumes of distribution (0.15–0.40 l/kg) approximate to extracellular fluid volume. Papich and Riviere provide an excellent summary of the many publications in this field.[27] Likewise, for cephalosporins, volumes of distribution generally approximate to extracellular fluid volume, and terminal half-lives are $\leq$2.0 h. An interesting exception is cefovecin, the half-life of which is 7 days in cats and 5 days in dogs. These high values are attributable mainly to a very high degree of binding to plasma protein, greatly limiting ultrafiltration at the glomerulus and presumably a lack of proximal tubular secretion, thus limiting renal excretion. Not surprisingly, this drug is not licensed for use in food-producing species. If its pharmacokinetic properties were similar to those in the dog and cat, the clearance from tissues would be unduly protracted.

It has been commonly assumed that, because of their lipophobic character and excretion in high concentrations in urine, β-lactams are metabolized slightly or not at all. This assumption is contraindicated (for amoxicillin) by the identification of metabolites, amoxicilloic acid, and amoxicillinpiperazine-2,5-lione, in tissues of pigs, after medication of drinking water with amoxicillin.[28] An important problem with frequently reported bio-analytical methods for amoxicillin is the use of a derivatization step during sample pre-treatment. Most derivatization procedures lead to the same reaction product for both amoxicillin and its amoxicilloic acid metabolite, with identical relative retention times during chromatography. This might result in an overestimation of the actual amoxicillin residue concentration.[29]

The pharmacokinetic profiles of β-lactams dictate tissue depletion profiles. Concentrations are generally high in the kidney, very low in fat, and also low in muscle. For example, in pigs, Martinez-Larrañaga et al. reported concentrations (mg/kg) of amoxicillin of 23.6 (muscle), 24.7 (fat), 49.1 (liver), and 559.7 (kidney) 2 days after oral dosing of 20 mg/kg orally for 5 days.[30] In broiler chickens administered amoxicillin in drinking water daily for 5 days, tissue concentrations (μg/kg) 1 h or less after final doses were 138 (muscle), 108 (fat), 484 (skin and fat), 2178 (liver), and 4363 (kidney).[31] For fat, the poor uptake is explained by both low bloodflow and the lipophobic character of the drugs. For muscle, the low concentration is related to poor intracellular penetration and hence restriction to the extracellular fraction of the tissue.

In food-producing species, there are compelling economic and welfare reasons for minimizing the number of AMD administrations in dosage regimens. The ideal is to achieve bacteriological and clinical cures with a single dose. For β-lactams, which are classified as time-dependent in their killing actions against most susceptible organisms, there is the additional requirement to maintain plasma drug concentrations in excess of MIC for at least half and indeed possibly for the whole of the inter-dosing interval, namely, not allowing concentrations to decrease below MIC until bacteriological cure is achieved. To attain this goal with intravenous dosing of β-lactams is, under clinical conditions, wholly impractical. One solution has been to use, instead of water-soluble sodium and potassium salts of penicillins, less soluble organic salts, such as procaine, benethamine, and benzathine benzylpenicillins.

Benzathine salts have particularly low aqueous solubility and, when injected intramuscularly, form a depot from which dissolution occurs slowly. Indeed, all benzathine benzylpenicillin salts have been banned from use in the food-producing animals in the EU, because of persistence at and erratic rate of depletion from injection sites and a consequent perceived hazard to human health. Procaine salts, on the other hand, are somewhat more water-soluble and remain in widespread use, formulated as aqueous or oily suspensions. These formulations provide flip-flop pharmacokinetics with terminal half-lives in the range of 8.9–17.0 h after intramuscular or subcutaneous dosing to calves and adult cattle. In some studies, absorption

rate (reflected by terminal half-life) was slower after subcutaneous dosing compared to intramuscular dosing. There are reports, indicating differences in absorption rate for different muscle groups, that absorption from neck muscle is slower than that from gluteal muscle.[32,33] These formulations are designed for once-daily dosing regimens. Other depot formulations have been developed, for example, using aqueous suspensions of ampicillin and amoxicillin trihydrates.

Another major clinical use of β-lactams is for intramammary treatment of bovine mastitis, in both lactating and dry cows. The lactating cow (see Table 2.3 for AMD milk : plasma concentrations in lactating cows) products are administered in rapid-release formulations to achieve milk concentrations often greatly in excess of MIC_{90} values for susceptible bacteria. These products are usually rapidly cleared from the gland after two or three infusions, providing short milk withholding periods. The dry-cow formulations are administered at dry off in fixed oil formulations and sometimes containing water repellent agents, such as aluminum monostearate, to prolong presence in the gland for most or all of the dry period.

2.3.3 Quinoxalines: Carbadox and Olaquindox

Carbadox has been used as a feed additive in pigs, as a growth promoter, and therapeutically for the control of swine dysentery, enteritis, and nasal infections. Both drugs are absorbed after oral dosing, but published information on their pharmacokinetic profiles is limited. The major residue metabolite of carbadox is quinoxaline-2-carboxylic acid. After feeding carbadox to the pig (50 ppm) as a growth promoter, residues in liver and kidney exceeded 30 μg/kg for 4–5 weeks and 10 μg/kg at 62 days.[34] Carbadox is both mutagenic and carcinogenic, while olaquindox is mutagenic but probably not a carcinogen. While there is concern regarding the safety of residues, there is evidence to indicate that the residues do not possess mutagenic or carcinogenic activity. It has been suggested that any risk is likely to be to individuals handling products containing the drugs.[35] Carbadox and olaquindox were withdrawn as feed additives in the EU in 1999. In the United States, the marker residue for carbadox is quinoxaline-2-carboxylic acid and the tolerance for pig liver is 30 μg/kg. In Australia, the MRL for olaquindox in pig and poultry meat has been set at 300 μg/kg.

Anadón et al. described the residue pharmacokinetics of olaquindox in broiler chickens (Table 2.4).[36] Absorption was rapid (T_{max} = 0.22 h) and terminal half-life was 5.13 h. Tissue depletion rates of olaquindox illustrate well the general principles for AMDs that (1) depletion rates are tissue-dependent and (2) peak concentrations (in this case in kidney) are not necessarily at the first slaughter time.

2.3.4 Lincosamides and Pleuromutilins

Three members of the lincosamide class, lincomycin, pirlimycin, and clindamycin, and two drugs in the pleuromutilin group, tiamulin and valnemulin, are used in veterinary medicine. The binding sites are similar to those of macrolides and, like macrolides, are lipophilic weak organic bases. As predictable from their weakly basic character, they achieve high concentrations in milk and intracellular fluid, and therefore tissue concentrations generally exceed those in plasma and interstitial fluid.

Lincomycin is formulated as a pre-mix and as a soluble powder for addition to drinking water for use in poultry and pigs and is also available in a parenteral formulation for the pig. Oral administration is contraindicated in all ruminants because of the risk of bacterial overgrowth with *Clostridium* species. However, lincomycin is licensed for use parenterally in calves as a combination product

TABLE 2.3 Milk : Plasma Concentration Ratios of AMDs in Lactating Cows[a]

Drug	Drug Class	Lipid Solubility	pK_a	Milk Ultrafiltrate : Plasma Ultrafiltrate	
				Theoretical Ratio	Experimental Ratio
Bases					
Trimethoprim	2 : 4 diaminopyrimidine	High	7.3	2.32 : 1	2.90 : 1
Spiramycin	Macrolide	High	8.2	3.57 : 1	4.60 : 1
Dihydrostreptomycin	Aminoglycoside	Low	7.8	3.13 : 1	0.50 : 1
Polymixin B	Polymyxin	Very low	10	3.97 : 1	0.30 : 1
Acids					
Benzylpenicillin	Penicillin	Low	2.7	0.25 : 1	0.13 : 1–0.26 : 1
Sulfadimethoxine	Sulfonamide	Moderate/high	6	0.20 : 1	0.23 : 1
Sulfamethazine	Sulfonamide	Moderate/high	7.4	0.58 : 1	0.59 : 1

[a]Note the poor penetration into milk of strong or polar bases, strong acids, and weak acids and good penetration of weak bases, except streptomycin, which is very polar as a result of the presence of sugar residues in the molecule.

Source: Adapted from Baggot et al. (2006).[152]

TABLE 2.4 Tissue Concentrations of Olaquindox after Oral Administration in Chickens (Mean ± SEM, $n = 6$)[a]

Tissue	Drug Concentration (mg/kg)				
	Day 1	Day 3	Day 6	Day 8	Day 14
Muscle	3.33 ± 0.84	1.69 ± 0.51	0.38 ± 0.08	0.18 ± 0.04	0.03 ± 0.01
Liver	3.69 ± 0.50	2.93 ± 0.38	1.49 ± 0.33	0.88 ± 0.22	0.11 ± 0.01
Kidney	1.43 ± 0.23	2.23 ± 0.65	1.92 ± 0.28	1.34 ± 0.18	0.12 ± 0.01

[a] Administered orally directly into the crop.

Source: Data from Anadón et al (1990).[36]

with spectinomycin for treatment of lung infections. The combination has also been used in sheep, goats, and poultry. In the pig, absorption from the GIT is rapid but bioavailability is limited, in the range of 20–50%. Lincomycin is well distributed into tissues, with relatively high concentrations obtained in liver and kidney. Muscle and skin concentrations, on the other hand, are low. Elimination is primarily through hepatic metabolism and approximately 20% of the administered dose is excreted in urine as parent drug. Diffusion trapping occurs in fluids and tissues, such as milk and the prostate, which are acidic relative to plasma. Volume of distribution is in the range of 1.0–1.3 l/kg.

In the chicken, after 7 days of oral dosing, feces and urine (combined) contained approximately 80% parent drug, 10% sulfoxide metabolite, and 5% *N*-demethyllincomycin.[37] The same authors reported excretion of 11–21% of the administered dose (half as parent drug) in urine. The remainder was excreted in feces, of which 17% was parent drug and 83% as uncharacterized metabolites.

Pirlimycin is used solely by intramammary infusion for the treatment of mastitis in lactating cattle.

Tiamulin is formulated as the base for parenteral use and as the hydrogen fumarate for oral use in drinking water and pre-mix soluble formulations. Valnemulin is also formulated as a pre-mix, as the hydrochloride salt. They are used in the pig against *Mycoplasma* lung infections and swine dysentery, in poultry for both *Mycoplasma* and *Brachyspira* infections, and to a lesser degree in treatment of calf pneumonia. The absorption of tiamulin is also high when administered orally as a bolus dose, but bioavailability is said to be lower from pre-mix formulations.[38] In calves the half-life is short (25 min), and after oral dosing absorption is rapid in pre-ruminant calves. Pleuromutilins are not used in calves with functional rumens. Concentrations in milk and lung tissue exceed those in plasma severalfold.

2.3.5 Macrolides, Triamilides, and Azalides

Drugs in this class include erythromycin, tylosin, spiramycin, tylvalosin, carbomycin, oleandomycin, tilmicosin (all macrolides), azithromycin (an azalide), and tulathromycin (a triamilide). The latter drug is a regioisomeric mixture of 13-membered (10%) and 15-membered (90%) ring compounds, while erythromycin is a mixture of three related compounds, named A, B, and C. Several drugs of this class are in widespread use in food-producing species. Carbomycin, oleandomycin, and tylosin have been used as pre-mixes for addition to the feed of poultry, pigs, and cattle, either as growth promoters or for disease prophylaxis and treatment. Tylosin and tulathromycin are used in parenteral formulations as therapeutic agents for calves and pigs, and tilmicosin is used in cattle only.

The weakly basic nature (pK_a 6–9) of macrolides results in partial ionisation at physiological pHs, but the un-ionized fractions possess moderate to high lipid solubility, so that they are well absorbed orally (except for erythromycin base) and readily penetrate into intra- and transcellular fluids. However, absorption may be impaired by feed. Erythromycin base is unstable in acid gastric juice and is therefore administered either in enteric-coated formulations or as estolate or ethylsuccinate esters or the stearate salt. These esters and the salt have improved bioavailability; the esters are hydrolysed after absorption. Their weakly basic character results in diffusion trapping in acidic fluids, such as milk (Tables 2.2 and 2.3). Volumes of distribution generally exceed body water volume, sometimes by a considerable amount. For example, the reported distribution volume of tylosin in calves ranging in age from 2 to >6 weeks is 9–11 l/kg.[39] Volumes of distribution of 20 and 11 l/kg have been reported, respectively, for azithromycin and tulathromycin. A characteristic of macrolide distribution is a strong tendency to concentrate intracellularly in some tissues, notably the lung and lung macrophages. This is probably attributable to their basic nature, reflecting the Henderson–Hasselbalch diffusion trapping mechanism and being due to the acidic pH in the cell phagolysosome. Plasma protein binding is relatively low, of the order of 18–30%.

The terminal half-life of erythromycin A in calves and adult cows is relatively short (2.9–4.1 h) after intravenous administration, but much longer after intramuscular (11.9 h) or subcutaneous (18.3 to 26.9 h) dosing, as a consequence of flip-flop pharmacokinetics of commercially available formulations, that is, of a very slow process of drug

absorption from its injection site. Concentrations in tissue (liver and kidney) and fluid (bile and prostatic) exceed those in plasma. Erythromycin is metabolized by demethylation in the liver by microsomal enzymes. Some 90% of the administered dose is excreted in bile, mainly as metabolites. No more than 5% is excreted in urine as the parent molecule.

After intravenous dosing, the half-life of tylosin is short in all species, 1.1, 2.1, 3.0, and 4.0 h, respectively, in calves, sheep, goats, and pigs.[40] Tylosin penetrates readily into milk and is slowly cleared from the mammary gland, so that its use in lactating cattle is not recommended. In fact, this property extends to other macrolides, due to their basic nature and lipophilic properties. For example, the half-life of tilmicosin in cows is approximately 1 h, but concentrations in milk exceed 0.8 mg/l for 8–9 days after a single subcutaneous dose of 10 mg/kg.

Tulathromycin is administered intramuscularly in pigs and subcutaneously in cattle. In both species bioavailability is of the order of 90% and volume of distribution is 1.2 l/kg. Tylvalosin, tilmicosin, and to an even greater extent tulathromycin among the macrolides achieve high concentrations in lung tissue. For the latter drug, lung : plasma concentrations exceeding 100 : 1 have been reported in the calf and the pig. The terminal half-life for lung tissue exceeds that of plasma; values for calves and pigs are 184 and 142 h, respectively, compared to serum half-lives of 90 and 76 h. Several macrolides, azalides, and triamilides have been shown to achieve high concentrations in leucocytes and off-loading of drug from polymorphonuclear neutrophils (PMNs) has been shown *in vitro* and proposed as a mechanism of delivery drug *in vivo* to the biophase. In fact, considering the rate of antibiotic efflux from PMN (rather slow), the total body pool of PMN (small relative to body weight), and using mass balance considerations, it is unlikely that neutrophils migrating preferentially to sites of infection provide a delivery mechanism for AMDs able to maintain, dynamically, a high local antibiotic concentration in the biophase that is extracellular water. In addition, using the microdialysis technique, it was shown that an acute inflammatory event seems to have little influence on tissue penetration. As quoted by Muller et al., "these observations are in clear contrast to reports on the increase in the target site availability of antibiotics by macrophage drug uptake and the preferential release of antibiotics at the target site, a concept which is also used as a marketing strategy by the drug industry."[41] The terminal half-life of tilmicosin is 1 h in the cow and 25 h in the pig.

2.3.6 Nitrofurans

In many countries, including the EU member states and the United States, the use of nitrofurans and furazolidone has been banned in food-producing animals, as they are genotoxic and furazolidone is carcinogenic. Therefore, from a residue perspective the interest lies in their illegal use. They are lipid-soluble weak acids, well absorbed orally, and bioavailability is enhanced when administered with feed. Some 50% of administered dose is metabolized, and the remainder is excreted in urine. In acid environments they are un-ionized, so that acidification of the urine promotes renal reabsorption and alkalinization enhances excretion.

2.3.7 Nitroimidazoles

Nitroimidazoles are antibacterial and antiprotozoal drugs, of moderate to high lipid solubility, and high bioavailability in monogastric species. The principal members of the group are metronidazole, tinidazole, ronidazole, and dimetridazole. They were used formerly in poultry and game birds to treat histomoniasis and *Spironucleus* infections and also swine dysentery in pigs. They have been classified as suspect mutagens and carcinogens,[42,43] and all the compounds in the group, except metronidazole and tinidazole have been removed from the market. All nitroimidazoles have been prohibited from use in food-producing animals in the United States and EU, where they are placed in Annex IV[44] (see Section 2.5.3).

2.3.8 Phenicols

The phenicol group of AMDs includes chloramphenicol, florfenicol, and thiamphenicol. All phenicols are relatively small organic molecules, containing neither acidic nor basic groups, and all are very lipid-soluble.

The original member of the group, chloramphenicol, has been used therapeutically for more than 60 years, and its pharmacokinetics have been studied extensively in many species, including food-producing animals. However, its toxicity profile includes a very rare but fatal form of aplastic anaemia in humans (incidence 1 : 10,000–1 : 45,000), which is not concentration-dependent. Therefore, in the United States, EU, Canada, Australia, and indeed in most jurisdictions, chloramphenicol is classified as a drug with a risk to public health and its use has been banned in food-producing animals. However, from an EU perspective, chloramphenicol continues to be used legally or illegally in some countries, and controls are still required, especially for imports of animals and their products from third-world countries (honey, crab meat, etc.). For chloramphenicol (as for nitrofurans), which has been expressly prohibited from use in food-producing animals in the EU, the concept of minimum required performance limit (MRPL) has been established in the Commission Decision 2002/657/EC.[45] MRPLs are defined as "minimum content of an analyte in a sample, which at least has to be detected and confirmed" and are the reference points for action in relation to the evaluation of consignments of food. To date, MRPLs have been

established for chloramphenicol of 0.3 and 1 μg/kg for nitrofurans.[46]

After intravenous dosing, chloramphenicol clearance in ruminant species is rapid and the terminal half-life is short: 1.7 h in sheep, 1.2–4.0 h in goats (the longer time $T_{1/2}$ in goats is after a period of food deprivation), and 2.5–7.6 h in calves. The longer time period is seen in young calves (7.6 h at 1 day and 4.0 h at 14 days) than in animals aged 9 months (2.5 h).[40] The half-life is also longer in piglets (12.7–17.2 h) than in adult pigs (1.3 h). In piglets the shorter half-life (12.7 h) was obtained in colostrum-fed animals, and the longer half-life (17.2 h) occurred in colostrum-deprived piglets. In the chicken, half-life was much longer in *E. coli*–infected animals than in healthy animals: 26.2 h compared to 8.3 h.[40]

Chloramphenicol is well absorbed after oral dosing in ruminants but is rapidly inactivated by ruminal microflora, so that bioavailability is extremely low. Its distribution in the body is widespread; as predicted from its lipid-soluble character, it readily penetrates into intracellular and transcellular fluids and readily diffuses into milk. Plasma protein binding is of the order of 30–45%. The volume of distribution is of the order of 1.0–2.5 l/kg. Urinary excretion in calves is minimal. Elimination is attributable primarily to metabolism in the liver, for example, by hydrolysis (phase I) and by glucuronidation (phase II) reactions. A range of metabolites has been identified, including dehydrochloramphenicol, nitrophenylaminopropanedione, and nitrosochloramphenicol.[47] These authors reported that the latter two compounds were still detectable in kidney, liver, and muscle of chickens at 12 days post-slaughter after oral dosing with chloramphenicol at 50 mg/kg daily for 4 days. Its rapid clearance and short half-life necessitate administration with a short dosing interval.

Thiamphenicol is a semi-synthetic derivative of chloramphenicol. It can cause reversible bone marrow depression, but fatal aplastic anemia has not been reported in humans. Oral bioavailability in pre-ruminant calves is 60%. It is somewhat less lipid- and somewhat more water-soluble than chloramphenicol and therefore crosses cell membranes less readily. Hepatic metabolism is limited, and elimination is primarily as parent drug in the urine. Limited published data indicate that it has a high distribution volume in ruminants. It has been used "in feed" in pigs and chickens, but such usage is now limited.

As a successor to chloramphenicol, florfenicol is now used extensively in food-producing species, particularly calves, chickens, and young pigs. It lacks the *para*-nitro group of chloramphenicol, which seems to be an essential molecular feature for causing aplastic anaemia. Therefore, there is no public health risk relating to aplastic anaemia arising from the use of florfenicol.

Florfenicol, like chloramphenicol, is very lipid soluble and is well absorbed in calves after oral dosing (bioavailability of 79–89%) but with some reduction in bioavailability when administered with milk. Bioavailability is also high from intramuscular and subcutaneous injection sites. The terminal half-life after intravenous dosing in calves is short (2.7–3.7 h), but as a consequence of slow absorption and flip-flop pharmacokinetics it is much longer (18 h) after intramuscular dosing. The clinically recommended intramuscular dose is 20 mg/kg. When florfenicol is administered to calves subcutaneously at the higher dose rate of 40 mg/kg, terminal half-life is even longer, so that effective therapy can often be achieved with single-dose administration. In the fish species, red pacu and salmon, half-lives were 4.3 and 12.2 h, respectively. The latter value was determined at 10.8°C. In rainbow trout, the mean residence time at 10°C was 21 h.

Florfenicol is widely distributed, achieving high concentrations in muscle, kidney, urine, milk, bile, and small intestine, but with lesser penetration of the blood–brain barrier than chloramphenicol. Volume of distribution in calves is similar to body water volume (0.67–0.91 l/kg), and binding to plasma protein is low (13–19%). Approximately two-thirds of the administered dose is excreted in calf urine as parent drug. The biologically inactive metabolite, florfenicol amine, is eliminated more slowly than parent drug and is used as the marker residue with the liver as the target tissue in some jurisdictions. For example, Anadón et al. recorded highest residue concentrations in the chicken in liver, with lower and similar residue depletion profiles in kidney, muscle, and skin plus fat.[48] For florfenicol amine, residue depletion profiles were similar for kidney and liver, with much lower concentrations in muscle and skin plus fat. In the EU the marker residue is the sum of florfenicol and all metabolites expressed as florfenicol amine.

Florfenicol is available in a range of formulations: in two strengths for parenteral administration in pigs and cattle, as a solution for addition to drinking water in pigs, and as pre-mixes for incorporation into feed for pigs and fish.

2.3.9 Polyether Antibiotic Ionophores

This is a unique class of compounds with high potency against a range of critical infectious disease targets, including protozoa, bacteria, and viruses. The principal drugs in this class are lasalocid, maduramicin, monensin, narasin, semduramicin, and salinonycin. All are coccidiostats, with widespread use in poultry. As a consequence of species-based toxicity, they are not used in horses and guinea fowl, and salinomycin and narasin are not used in turkeys. For some ionophores, toxicities may be exacerbated when administered in combination with erythromycin, tiamulin, pleuromutilins, sulfonamides, and chloramphenicol, as a consequence of inhibition of ionophore metabolism.

All polyether ionophores are formulated for use in chickens in feed for the prevention of coccidiosis. Some are also licensed for use, in feed, as coccidiostats in goats (monensin), cattle (lasalocid, monensin), sheep (lasalocid), rabbits (lasalocid), turkeys (lasalocid, monensin), chukar partridges (lasalocid), and bobwhite quail (monensin, salinomycin). For individual drugs there are various restrictions on use, including narasin, for use in broiler chickens only, and monensin, which is not for use in goats producing milk for human consumption. Some ionophores are added to animal feeds as growth promoters for use in pigs and/or cattle. However, such use has been disallowed in the EU since 2006. Monensin is also licensed for improved milk production in cattle. It might be noted that there is a possibility of carryover of drugs of this class from non-target animal feed, which might give rise to residues in animal products for which no MRLs are set.[49,50]

Published data on the pharmacokinetics of ionophores are limited. Dowling reports high bioavailability in monogastric species and approximately 50% in ruminants.[51] Most ionophores are metabolized extensively in the liver, forming many metabolites that are secreted in bile and excreted in feces.

2.3.10 Polypeptides

The drug groups in this general category are polymyxins (see Section 2.3.13), glycopeptides, bacitracin, and streptogramins. The principal members of the glycopeptide group are vancomycin, teicoplanin, and avoparcin. The former two drugs are used in human therapeutics, and avoparcin has been used extensively as a growth promoter in poultry and pigs, particularly in the EU. However, it has been withdrawn from use in the EU, because of selection for vancomycin-resistant enterococci (VRE) in farm animals, which may potentially transfer resistance to human pathogens. This is of concern because vancomycin is a drug of last resort for serious human infections caused by drug-resistant Gram-positive bacteria. It might be noted that VRE cause significant problems in human hospitals in North America, where avoparcin has never been used in animals. In Australia avoparcin retains an MRL listing. Vancomycin has been used, by intravenous infusion, in horses and dogs but not in farm animal species. Its use in food-producing animals was banned in the United States in 1997.

Vancomycin is a high-molecular-weight polypeptide and teicoplanin is similar in structure and, in fact, is a complex of five related compounds. Both are poorly lipid-soluble. For both compounds, absorption after oral dosing is slight/absent, a property relating to the low lipid solubility and polypeptide structure, as they are broken down to constituent amino acids in the GIT. Teicoplanin is well absorbed after intramuscular dosing, and has a prolonged half-life in humans of 45–70 h. Vancomycin is too irritant for intramuscular administration. It is, therefore, administered intravenously in humans, where the terminal half-life is 6 h. Both drugs are poorly distributed, restricted primarily to extracellular fluids, and excreted largely unchanged by glomerular ultrafiltration.

Bacitracin has been administered orally as a growth promoter in poultry and pigs (although this use is no longer permitted in the EU) and as a therapeutic for enteritis, although it is not effective in swine dysentery. Absorption from the GIT is very low, which is fortunate as bacitracin is nephrotoxic.

Streptogramins are natural (e.g., virginiamicin) or semi-synthetic (e.g., quinupristin/dalfopristin) cyclic peptides. Virginiamycin has been used as a growth promoter. It is the only member of the group used in animals and is a mixture of two compounds, virginiamycin S (a cyclic hexadepsipeptide, the minor component) and virginiamycin M (a macrolactone, the major component). The use of virginiamycin as a feed additive in pigs and poultry can result in the selection of resistance in fecal enterococci with cross-resistance to quinuspristin/dalfopristin, which has been used in human medicine to treat VRE infections. Virginiamycin is poorly absorbed after oral dosing and is excreted in bile. It was banned as a growth promoter for pigs in the EU in 2009 but is still used for this purpose in some countries. It has also been used as a therapeutic agent in swine dysentery and laminitis in horses.

2.3.11 Quinolones

First-generation quinolones were nalidixic and oxolinic acids. The latter is still used therapeutically in fish, but otherwise these drugs have been superseded by the fluoroquinolone sub-group. The principal fluoroquinolones used in food-producing species are danofloxacin, enrofloxacin, flumequine, marbofloxacin, and sarafloxacin. They contain both carboxylic acidic and basic amino groups and are therefore amphoteric; pK values for the former are 5.5–6.5 and for the latter 7.5–9.3, so that at physiological pH they exist as zwitterions (partially ionised, partially un-ionized for each group). The drugs are most lipophilic at the isoelectric point, which is close to blood pH. Lipophilicity varies between drugs but is always moderate (ciprofloxacin, marbofloxacin) or high (enrofloxacin). Two members of the group, enrofloxacin and sarofloxacin, were formerly used in poultry but have now been banned in the United States and Australia because of concerns about *Campylobacter* and *Salmonella* resistance. Many other fluoroquinolones are used extensively in human medicine.

The pharmacokinetic profiles of danofloxacin, enrofloxacin, flumequine, and marbofloxacin in food-producing species have been studied extensively.[52] Bioavailability for all drugs in all species is very high after intramuscular dosing. Some studies in calves have

demonstrated flip-flop pharmacokinetics after intramuscular and subcutaneous dosing. Binding to plasma protein is relatively low to moderate and varies with species for enrofloxacin; it is low in the pig (27%) and chicken (21%) and moderate in cattle (36–60%). Volumes of distribution are of the order of 1.0–4.0 l/kg, that is, exceeding total body water volume, and elimination half-lives are in the range 2.0–8.0 h in cattle, sheep, goats, and pigs. Shorter half-lives in rabbits (1.8–2.5 h for enrofloxacin) and longer half-lives in fish (24 h in trout and 131.0 h in Atlantic salmon, both for enrofloxacin) and chickens (5.6–14.0 h for enrofloxacin) have been reported. Half-lives are also longer in reptiles (36 and 55 h for enrofloxacin in monitor lizards and alligators, respectively). For free plasma concentration of enrofloxacin, an allometric relationship seems to apply between volume of distribution and body weight, with a direct proportionality, specifically, larger distribution volumes in heavier animals.[52]

For pigs and ruminant species, dosing is generally by intramuscular injection, with once-daily dosing schedules. For example, Anadón et al.[53] reported a bioavailability of 74.5% after intramuscular dosing of enrofloxacin (2.5 mg/kg) in pigs, and tissue residue concentrations of enrofloxacin and the ciprofloxacin metabolite (mg/kg) at 5 days were 0.03, 0.08 (fat), 0.06, 0.04 (kidney), 0.06, 0.02 (liver), and 0.06, <0.003 (muscle). Products of higher strength in depot formulations have been developed for danofloxacin, enrofloxacin, and marbofloxacin and these provide, after intramuscular or subcutaneous injection, therapeutic levels for 48 h or longer; they are commonly administered as single doses. For most drugs, in most parenteral formulations, bioavailability is in the range of 75–100%.

Oral bioavailability of fluroquinolones is high in both presence and absence of feed, in most monogastric species, including the pig, but in ruminants, this administration route is not used. Nevertheless, data in adult sheep suggest good bioavailability (61%), whereas bioavailability from oral dosing was only 10% in ruminant calves. Enrofloxacin is well absorbed after oral dosing in poultry, but its use is not permitted in the EU for animals producing eggs for human consumption. Bioavailability from oral dosing of enrofloxacin in fish is reported to be 40–50%. In chickens selected for fattening, the oral bioavailability of flumequine was 57% after oral dosing.[54] These authors reported highest residue concentrations of flumequine and the metabolite 7-hydroxyflumequine in kidney, followed by liver, with lower concentrations in muscle and skin plus fat. Anadón et al. also described the differing residue depletion profiles of marbofloxacin and its *N*-desmethyl metabolite in broiler chickens.[55] In plasma at day 1 following oral administration, marbofloxacin and its *N*-desmethyl metabolite concentrations were 0.047 ± 0.003 mg/l and 0.032 ± 0.004 mg/l, respectively, but were not detectable on subsequent sampling dates. Residues found in edible tissues are given in Table 2.5.

TABLE 2.5 Residues of Marbofloxacin and *N*-Desmethylmarbofloxacin in Edible Tissues of Chickens Following Oral Administration of Marbofloxacin (2 mg/kg, every 24 hours, for 3 days)

Tissue	Days Post-treatment (withholding period)	Marbofloxacin (μg/g)	*N*-Desmethyl-marbofloxacin (μg/g)
Muscle	1	32 ± 3	119 ± 23
	2	18 ± 3	113 ± 23
	3	<LOD	<LOD
	5	<LOD	<LOD
Kidney	1	985 ± 72	499 ± 60
	2	420 ± 48	164 ± 32
	3	40 ± 4	69 ± 13
	5	7 ± 2	21.7 ± 4.9
Liver	1	735 ± 45	554 ± 66
	2	343 ± 38	158 ± 30
	3	28 ± 7	99 ± 15
	5	11 ± 2	51 ± 8
Skin plus fat	1	43 ± 6	266 ± 58
	2	10 ± 2	55 ± 10
	3	<LOD	<LOD
	5	<LOD	<LOD

Source: Data from Anadón et al (2002).[55]

The distribution of fluoroquinolones into interstitial fluid has been shown to be predictable from free concentrations in plasma.[56] Like the macrolides group of AMDs, fluoroquinolones achieve high concentrations in leukocytes. Concentrations in lung, liver, and kidney are several times higher than those in plasma.

For enrofloxacin, there is an additional consideration in relation to pharmacokinetic and residue profiles, in that it is metabolized in the liver to a microbiologically active metabolite, ciprofloxacin, by a deethylation reaction. In cattle and calf conversion rates, from enrofloxacin to ciprofloxacin, are 25% and 41%, respectively. Residues are measured as the sum of enrofloxacin and ciprofloxacin. In poultry, pigs, and fish, much smaller amounts of ciprofloxacin are formed. Nevertheless, in chickens, ciprofloxacin residues were detectable 12 days after dosing with enrofloxacin.[57] Ciprofloxacin itself is converted to minor metabolites with no antibacterial activity. Nevertheless, metabolites are of residue concern, and tissue depletion profiles were studied in broiler chickens by Anadón et al.[58] The data in Table 2.6 illustrate the rapid conversion of ciprofloxacin to oxociprofloxacin and desethyleneciprofloxacin (T_{max} < 1.0 h), the accumulation

TABLE 2.6 Residue Pharmacokinetics of Ciprofloxacin and Its Metabolites in Broiler Chickens after Oral Dosing of Ciprofloxacin[a]

	Ciprofloxacin			Oxociprofloxacin			Desethyleneciprofloxacin		
Variable	Day 1	Day 5	Day 10	Day 1	Day 5	Day 10	Day 1	Day 5	Day 10
Plasma concentration (mg/kg)	0.14 ± 0.02	N/D	N/D	0.10 ± 0.02	N/D	N/D	0.10 ± 0.02	N/D	N/D
Kidney concentration (mg/kg)	0.74 ± 0.07	0.69 ± 0.06	N/D	1.27 ± 0.13	0.63 ± 0.07	N/D	0.97 ± 0.27	0.23 ± 0.07	N/D
Liver concentration (mg/kg)	0.74 ± 0.22	0.55 ± 0.21	N/D	1.78 ± 0.72	0.75 ± 0.38	N/D	1.28 ± 0.62	0.59 ± 0.47	0.011 ± 0.008
Muscle concentration (mg/kg)	0.37 ± 0.06	0.020 ± 0.008	N/D	0.68 ± 0.20	0.32 ± 0.06	N/D	0.61 ± 0.26	0.35 ± 0.11	N/D
Skin+fat concentration (mg/kg)	0.23 ± 0.11	0.11 ± 0.06	N/D	0.51 ± 0.32	0.026 ± 0.011	N/D	0.95 ± 0.23	0.28 ± 0.08	0.010 ± 0.006
Plasma C_{max} (mg/l)		2.63 ± 0.20			1.73 ± 2.02			1.57 ± 0.14	
Plasma T_{max} (h)		0.36 ± 0.07			0.62 ± 0.08			0.75 ± 0.16	

[a] Administered at a rate of 8 mg/kg for 3 days (mean ± SD, $n = 6$).
[b] N/D not detectable.
Source: Data from Anadón et al (2001).[58]

of parent drug and both metabolites in kidney and liver, and the rates of depletion from all edible tissues.

The principal route of elimination of fluoroquinolones is via the kidney by glomerular filtration and, for some drugs, also by tubular secretion.[59] Smaller amounts are eliminated in feces.

2.3.12 Sulfonamides and Diaminopyrimidines

Sulfonamides are synthetic AMDs based on sulfanilamide as parent compound, which was introduced into medicine in 1935. Subsequently, large numbers of derivatives have been used clinically. In veterinary medicine sulfonamides are now used primarily in combination products containing the 2 : 4 diaminopyrimidines, trimethoprim and ormetoprim. The combinations are synergistic in their antibacterial actions. However, some sulfonamides (e.g., sulfadimethoxine, sulfanquinoxaline, sulfadimidine) are used alone in cattle and poultry as soluble powders or solutions for addition to drinking water or as extended-release tablets for cattle. For the latter, maintenance of therapeutic concentrations for 2–5 days has been claimed after a single dose. Overall, the potency of sulfonamides is low, so that high doses (20–100 mg/kg) are used therapeutically. This imposes a high metabolic load on the body and may saturate metabolic pathways, leading to dose dependence in clearance and terminal half-life.

As weak organic acids ($pK_a = 10.1$ for sulfanilamide, 6.1 for sulfadoxine), at physiological pHs of most body fluids they are mainly un-ionized. The un-ionized moiety is generally lipid-soluble, but this varies (lipophilicity high for sulfisoxazole, low for sulfaguanidine) between drugs. The consequence is that sulfonamides generally readily cross cell membranes and are diffusion/ion-trapped in fluids alkaline to plasma (e.g., intracellular fluid, alkaline urine). On the other hand, they penetrate poorly into fluids more acidic than plasma, such as prostatic fluid and milk (Table 2.3). Sulfonamides of high pK_a are generally the least water-soluble, and solubility in water is greater under alkaline than acidic conditions, so that the potential for precipitation to cause crystalluria and renal damage in acid urines has long been recognized, especially for those drugs of low potency and low water solubility. For sulfonamides of high pK_a, the percentage binding to plasma proteins tends to be low. Protein binding thus ranges from high (90% for sulfadimethoxine in some species) to as low as 15%. Moreover, binding to protein can vary considerably between species. Formerly used extensively were triple sulfonamide formulations, which were additive in their antimicrobial actions but obeyed the law of independent solubilities, enabling the use of lower doses of each drug in the combination.

Diaminopyrimidines are lipid-soluble weak organic bases, partially ionized at physiological pH, which, in contrast to sulfonamides, penetrate readily into cells and are poorly reabsorbed from acid urines.

As weak organic acids, sulfonamides are generally well absorbed after oral dosing in monogastric species, but rate and extent of absorption vary with species, drug (greater bioavailability of more lipid soluble drugs), and feed. For example, in horses the absorption of sulfachlorpyridazine was reduced, delayed, and exhibited two peaks when administered orally in the presence of food.[60] The double-peak phenomenon is likely due to partial binding of drug to feed by adsorption, with initial rapid absorption of an unbound fraction and subsequent absorption of the bound fraction following its release by fermentative digestion in

the large intestine. Those sulfonamides with very low lipid solubility (e.g., sulfaguanidine) are excreted unchanged, with only slight absorption, in feces when administered orally. They were formerly widely used to treat GIT infections.

An influence of disease on the absorption of sulfaquinoxaline was established by Williams et al.,[61] who reported a 3.5-fold greater bioavailability in chickens infected with *Escherichia acervalina* and *E. tenella* in comparison with uninfected birds.

Both age and diet may influence sulfonamide absorption in calves. Oral absorption of sulfadiazine was very slow in calves on milk diets, and bioavailability was greater in ruminating than milk-fed pre-ruminant calves.[62] Trimethoprim, on the other hand, was well absorbed in pre-ruminant calves but not in ruminating animals, possibly as a result of inactivation by ruminal microflora.

Sulfonamides are generally well distributed into extra- and transcellular fluids, but penetration into intracellular fluid is poor to moderate, a consequence of their acidic nature and the overall acid pH within cells.

A major metabolic pathway for sulfonamides is acetylation of the amino group in the *N*-4 position of the benzene ring. It occurs primarily in the liver but also in the lung. Acetylation is of interest for several reasons: (1) it generally occurs more rapidly in herbivores than in omnivores and carnivores—acetylated derivatives are the major urinary metabolites in cattle, sheep, and pigs; (2) it is species-dependent, in that it occurs to only a slight extent in chickens and dogs; and (3) the acetylated derivatives are commonly less water-soluble (and especially so in acidic fluids) than parent compounds, potentially leading to the condition of renal crystalluria. Those sulfonamides containing a pyrimidine ring (sulfamethazine, sulfamerazine, sulfadiazine) undergo hydroxylation of a methyl group within the ring. Other metabolic pathways include glucuronidation, sulfate conjugation, aromatic hydroxylation, and deamination. All known metabolites have either much reduced or no antimicrobial activity.

Sulfonamide excretion occurs partly via the parent compounds in urine (most readily if urine pH is alkaline, as in herbivores), but predominantly through the less lipid-soluble and therefore more readily excreted metabolites described above. Some sulfonamides are also excreted via the active carrier-mediated transport system, which secretes organic acids from peritubular capillaries across proximal convoluted tubule cells and into tubular lumen fluid. Acetylated sulfonamides are usually less water-soluble than the parent compounds and are the main cause of the crystalluria that can occur, leading to tubular damage. Only small amounts are excreted in bile and milk.

A summary of the detailed information on the pharmacokinetics of sulfamethazine (also known as *sulfadimidine*) and sulfadiazine is provided by Papich and Riviere.[63] Volumes of distribution of these drugs are low to moderate in most species (0.24–0.90 l/kg), but with the buffalo (V_d = 1.23 l/kg) and rainbow trout (V_d = 1.2 l/kg at 10°C and 0.83 l/kg at 20°C) as exceptions. In cattle, the elimination half-life ranged from 3.6 to 5.9 h with some evidence of age variability.[64] In goats, sulfadimidine half-life was of the same order as that of cattle, but with a longer half-life in fasted adults (7.03 h) than in fed adults (4.75 h).[65] Similar values were reported for ewes, but with a shorter terminal half-life, after oral dosing, with a low (100 mg/kg) compared to a high (391 mg/kg) dose, of 4.3 and 14.3 h, respectively.[66] In pigs, the half-life of sulfadimidine ranged from 11.9 to 20.0 h, with little dependence on age.

For sulfadiazine, Nouws et al.[67] reported long elimination half-lives in carp of 47.1 h at 10°C and 33.0 h at 20°C. However, as for sulfadimine, the elimination half-life of sulfadiazine in calves was in the range of 3.4–7.0 h, with no apparent relationship to age.[64] Sulfadimethoxine is a long-acting sulfonamide, with an elimination half-life of 12.5 h in calves,[68] 16.2 h in pigs aged 1–2 weeks, and 9.4 h in older (11–12 weeks) animals.[69] Mengelers et al. reported similar half-lives of approximately 13 h in healthy and febrile (inoculated endobronchially with *A. pneumoniae* toxins) pigs.[70] In sheep, the elimination half-life of sulfamerizine was longer (9–14 h) in lambs aged 1 week than in older animals (4–7 h) aged 9–16 weeks.

In several species, including calves and pigs, the distribution volume of trimethoprim is of the order of 1.8–4.0 l/kg, thus significantly exceeding body water volume, and reflecting ready penetration (as a weak base) into intracellular fluid to achieve high tissue concentrations. In calves aged 1–13 weeks, elimination half-life was in the range of 0.9–4.4 h, with no apparent relationship to age.[62] After intravenous dosing in pigs, half-life was 3.3 h. In the same study longer terminal half-lives of 6.5 and 10.6 h in fasted and fed pigs, respectively, after oral dosing, indicate the likelihood of flip-flop pharmacokinetics.[71] Nouws et al. reported long elimination half-lives of trimethoprim in carp of 40.7 and 20.0 h, respectively, at 10°C and 20°C.[67]

Several groups have reported the highest tissue concentrations of sulfonamides plus metabolites in liver and kidney of various food-producing species.[72] Tissue residues of sulfonamides have been described as a cause of special concern in several jurisdictions as they (principally sulfadimidine) have been the cause of more residue violations than any other AMD group, notably in the pig.[63] For example, an early report indicated that sulfadimidine and metabolites were the most frequent cause of non-compliant residues in pig meat, associated with its use as a feed additive.[73] After in feed dosing in pigs, high concentrations of sulfadimidine and metabolites were measured in liver and kidney, with low concentrations in fat.[74] The concern has been exacerbated by reports that sulfadimidine may be carcinogenic in mouse and rat studies. Bevill reported

(1) as a major contributory factor to sulfadimidine residues its relatively long terminal half-life of 12.7 h in the pig and (2) as major causes of violations, failure to observe the WhT, improper feed mixing, and inadequate cleaning of feed-mixing equipment, leading to cross-contamination of feed.[75] Accidental exposure, such as during transport, can also lead to the presence of non-compliant residues in tissues of pigs at slaughter.[76] The high rate of sulfonamide-related violations of pig kidney of 13% in the late 1970s has since decreased considerably.

2.3.13 Polymyxins

Of several polymyxins isolated and investigated (A, B, C, D, E, and M) only compounds B and E are in veterinary use, both as sulfate salts. Polymyxin B is a mixture of B_1 and B_2. Of greater clinical use is polymyxin E, more commonly known as *colistin*, and used as colistin methanesulfonate. Their cationic structure accounts for their disruptive interaction with cell membrane phospholipids and has been described as a detergent-like action. Polymyxins, which are highly ionized molecules, are polar and very poorly lipid-soluble.

As predictable from their very low lipid solubility, clearance is relatively rapid, involving excretion by glomerular ultrafiltration and rapid excretion in urine, although ultrafiltration is somewhat limited by relatively high binding (70–90%) to plasma protein. Colistin is excreted virtually unchanged, and terminal half-life is of the order of 3–4 h. In sheep, a volume of distribution of 1.29 l/kg has been reported. Absorption from the GIT is virtually absent after oral dosing. Because of well-defined neurotoxic and renotoxic properties, polymyxin B is not administered by any route that provides measurable plasma concentrations. However, polymyxin B is used as an endotoxin-neutralizing agent in some veterinary vaccines at doses not exceeding 60 μg/dose, and this does not raise safety (including residues) issues.

For colistin sulfate, no evidence of neurotoxicity was observed in experimental animals for doses much higher than therapeutic doses, and there are many colistin products available for parenteral and intramammary administration. Colistins have, in addition to bactericidal activity against Gram-negative bacteria, direct antiendotoxaemic actions through their binding capacity for anionic lipid, a portion of the endotoxin molecule. The main clinical use of colistin sulfate in food-producing animals is oral administration for the treatment of colibacillosis in young piglets. As absorption from the GIT is very low, residues in edible tissues are not considered to be a major concern. However, it might be noted that, like aminoglycosides, systemically available polymyxins become firmly bound to renal tissue and depletion from the kidney is very slow.

2.3.14 Tetracyclines

Drugs of the tetracycline group are amphoteric, forming salts with both acids and bases. They are used as parent compounds (e.g., oxytetracycline dihydrate) or as salts (e.g., oxytetracycline hydrochloride). Their lipid solubilities range from moderate (oxytetracycline and chlortetracycline) to high (doxycycline and minocycline), so that they are able to traverse cell membranes moderately or readily. The former two drugs are natural tetracyclines, while the latter two are semi-synthetic.

After oral dosing, the bioavailability of tetracyclines varies between drugs, being lowest for oxytetracycline and chlortetracycline and highest for doxycycline, but for all drugs except doxycycline, it is relatively low (Table 2.7). This is of importance from both therapeutic and residue perspectives, because low bioavailability is associated with a high degree of inter-animal variability in both amount absorbed and the resulting plasma concentration–time profile. This can be expected to lead to high variability between animals in residue depletion.

After absorption, the tetracyclines are partially bound to plasma protein. Reported values in farm animal species are 46–51% (chlortetracycline), 28–41% (tetracycline), 21–76% (oxytetracycline), and 84–92% (doxycycline).[77] For the latter drug, high protein binding raises questions concerning effective dosage. The recommended dosage for pigs in drinking water is 10 mg/kg, which provides $AUC_{24\ h}$/MIC ratios that are claimed to be effective for several respiratory tract pathogens.[78] However, Toutain and coworkers, cited by Lees et al.,[9] taking an $AUC_{24\ h}$/MIC breakpoint of 24 h (i.e., an average plasma concentration over the dosing interval equal to the MIC) and using population pharmacokinetic data, predicted for systemic effect a dosage of 20 mg/kg, based on total plasma concentration. Allowing for 90% protein binding, the

TABLE 2.7 Oral Bioavailability of Tetracyclines (Mean Values of Studies Reported)

Drug	Species	Systemic Bioavailability (F%)
Chlortetracycline	Chicken	1
	Turkey	6
	Pig	6, 11, 19[a]
Oxytetracycline	Pig	3–5
	Fish	6
	Turkey	9–48
Tetracycline	Pig	5, 8, 18, 23[a]
Doxycycline	Pig	21.2
	Calf	70
	Chicken	41.3
	Turkey	25, 37, 41, 63.5[b]

[a]These F% values are study- and feed-dependent.
[b]These F% values are age-dependent.
Source: Adapted from Papich and Riviere (2009).[21]

predicted effective dose would be 200 mg/kg, which is totally unrealistic.

Tetracyclines are used extensively in food-producing species. Thus, chlortetracycline, oxytetracycline, and doxycycline are formulated for use as both in-feed and/or in-water products, in poultry, pigs, fish, and cattle for some or all of the following purposes: growth promotion, prophylaxis, metaphylaxis, and therapy. Another major use, particularly of oxytetracycline in parenteral formulations, is for therapy of a range of diseases, including calf and piglet pneumonias. Parenteral solution formulations of various strengths, ranging from 5% to 30% and containing a range of organic solvents, such as propylene glycol, 2-pyrrolidone, and *N*-methylpyrollidone, are in widespread use. When used in higher strengths ($\geq$10%), the formulations create a depot, from which slow absorption occurs, at intramuscular injection sites. After intramuscular administration, a fraction of the dose that remains in solution is rapidly absorbed to achieve maximum plasma concentrations within 1–2 h. However, as the organic solvents in the formulation are dispersed and absorbed, a larger fraction of the administered oxytetracycline precipitates. This provides a depot for subsequent slow absorption phases, giving rise to flip-flop pharmacokinetics and also an acute inflammatory reaction (see Section 2.7.1).

There have been many studies in calves and pigs confirming the retardation effect (prolonged absorption) of high-dose, high-strength solutions of oxytetracycline. Craigmill et al. reported an analysis for 41 datasets from 25 published articles on oxytetracycline in cattle.[79] Their meta-analysis for a dose of 20 mg/kg intramuscularly indicated mean values of 5.61 μg/ml (C_{max}) and 21.6 h ($T_{1/2}$). The advantages of these formulations relate to convenience and economy (single injection) plus animal welfare (avoiding multiple injections) and maintenance of plasma concentrations equal to or greater than MICs of sensitive organisms for periods of 48–96 h. Nouws studied both irritation at injection sites and persistence of oxytetracycline for relatively long periods after intramuscular dosing with 10 of the then available formulations.[80] Injection site issues are considered in Section 2.7.1.

As tetracyclines have moderate to high lipophilic properties, the poor bioavailability associated with oral administration is somewhat surprising. Papich and Riviere suggest that causes may be multifactorial.[77] As zwitterions, they are mainly ionized at pHs within GIT liquor. Moreover, feed reduces bioavailability, and tetracyclines chelate with polyvalent cations. Oxytetracycline absorption has been shown, experimentally, to be reduced by feed, dairy products, Ca^{2+}, Mg^{2+}, Al^{3+}, and Fe^{2+} ions and antacids. Even though doxycycline has a similar structure, affinity for metals is different from that of oxytetracycline with greater affinity for zinc and less for calcium. Supplementation of feed for piglets with zinc can drastically reduce bioavailability of doxycycline.

Moderate variability in absorption of oxytetracycline from different intramuscular injection sites in calves was reported by Nouws and Vree.[81] Bioavailability values of 79%, 86%, and 89% were obtained for injection into the buttock, neck, and shoulder, respectively. The same group reported variable bioavailability and residue profiles with 10 formulations of oxytetracycline in pigs[80] and 5 formulations in calves, sheep, and pigs.[82]

Despite moderate to high degrees of binding to plasma protein, tetracyclines are generally well distributed to most tissues. Volumes of distribution are generally similar to body water volume (0.6–0.7 l/kg). Distribution volumes in excess of this are probably indicative of higher concentrations in intra- than extracellular fluid or binding to specific tissues, including bone. Doxycycline and minocycline traverse cell membranes better than do chlortetracycline and oxytetracycline, and doxycycline in particular concentrates intracellularly.

Systemic clearance of tetracyclines is similar to or higher than GFR. Up to 60%, depending on individual drugs, is eliminated by glomerular ultrafiltration and approximately 40% of administered dose is excreted in feces, but percentages are dependent on drug and route of administration. Bile : plasma concentration ratios may be as high as 20 : 1. For doxycycline, biliary excretion exceeds urinary excretion. Tetracyclines are also metabolized to inactive compounds, except for doxycycline, for which no metabolites have been detected in calves and pigs. In addition to possible metabolism, residue analysis, especially of chlortetracycline, can be hindered by the fact that chlortetracycline is subjected not only to epimerization but also to keto-enol tautomerism, resulting in keto-enol tautomers in the chromatogram, which influence the quantification of residues.[83] In the EU, MRLs for tetracyclines are expressed as the sum of parent compound plus the 4-epimer.

Terminal half-life varies with species, individual drug, and formulation. With the exceptions of retard, in-feed, and in-water formulations, the half-life in most species is sufficient to justify dosing once or twice daily. There are, however, exceptions; half-lives of oxytetracycline after intravenous dosing of 0.7 h (turkey) and 81.5 h (rainbow trout) have been reported.[77] As with all AMDs, there is the possibility of altered pharmacokinetics, as a consequence of disease, but the nature and direction of the change are not readily predictable. Pijpers et al.[84] reported an increase in half-life of oxytetracycline after oral dosing in pigs with pneumonia (14.1 h) compared to healthy pigs (5.9 h), with both values higher than half-life after intravenous dosing (3.7 h), described by Mevius et al.[85] In contrast, more recent studies in our laboratory indicate a lower AUC for oxytetracycline in pneumonic calves compared to healthy

animals.[86] Others have reported an increased volume of distribution in diseased animals.

Bound residues of tetracyclines may occur in bones of slaughtered animals for months after treatment. Theoretically, these could reach the food chain via contaminated (mechanically deboned) meat or meat and bonemeal. The accumulation of tetracyclines in tissues is illustrated by the findings of Toutain and Raynaud for oxytetracycline in calves (Table 2.8).[87] Concentrations of oxytetracycline were relatively high in liver and kidney compared to the extrapolated zero-time concentration for serum (4.2 mg/l). The time required for residues to deplete to 0.1 mg/l in serum was 143 hr, considerably shorter than the time required for residues to deplete to 0.1 mg/kg in liver and kidney, but similar to the depletion time for muscle. The data nicely illustrate the importance of tissue elimination half-life in determining decrease to the 0.1 mg/kg concentration; despite an almost three-fold higher initial concentration in kidney compared to liver, the longer half-life for liver leads to a longer time to depletion to 0.1 mg/kg for liver. Similar data were reported for doxycycline in broiler chickens.[88] After dosing orally at 20 mg/kg for 4 days, 1- and 5-day residue concentrations (mg/kg) were as follows: kidney 1.92 and 0.17, liver 1.93 and 0.12, and muscle 1.18 and 0.06, respectively. Other tetracyclines, including tigecycline, recommended for human but not veterinary use.

2.4 SETTING GUIDELINES FOR RESIDUES BY REGULATORY AUTHORITIES

All advanced and several emerging economies have well-established, legally binding procedures for evaluating applications for marketing authorizations (MAs) for veterinary medicinal products (VMPs). The principal bodies and their legal status are indicated in Table 2.9. In the case of the EU of 27 member states, as well as the supranational

TABLE 2.8 Oxytetracycline Residues in Calves after Intramuscular Administration of a Long-Acting Formulation[a]

Tissue	B_0[b] (mg/kg)	Tissue : Serum Ratio at Zero Time[c]	$t_{1/2\beta}$[d] (h)	Delay to Depletion to 0.1 mg/kg (h)
Liver	10.7	2.4 : 1	42.4	287
Kidney	28.9	6.4 : 1	23.6	193
Muscle	3.9	0.9 : 1	26.2	138

[a]Formulation: 20 mg/kg of a 20% w/v solution.
[b]Extrapolated zero-time concentration.
[c]The initial concentration in serum B_0: 4.5 mg/l.
[d]Elimination half-life.
Source: Data from Toutain and Raynaud (1983).[87]

TABLE 2.9 Major Regulatory Authorities Granting Marketing Authorizations for Antimicrobial Drugs[a]

Country	Authority	Acronym	Legal Basis
United States of America	Food and Drug Administration Center for Veterinary Medicine	FDA/CVM[b]	Federal Food, Drug and Cosmetic Act 1996, as amended, and associated regulations
European Union of 27 member states[c]	Committee for Medicinal Products for Veterinary Use, European Medicines Agency[d]	CVMP/EMA	EC Directive 2001/82/EC and Regulation 726/2004 of European Parliament and of Council as amended by Directive 2004/28/EC (*EUDRALEX* Vol. 5)
New Zealand	New Zealand Food Safety Authority	NZFSA/ACVM	Agricultural Compounds and Veterinary Medicines Act (ACVM)
Australia	Australian Pesticides and Veterinary Medicines Authority	APVMA	Agricultural and Veterinary Code Act 1994 (Agvet Code)
Japan	Pharmaceutical Affairs and Food Sanitation Council of the Ministry of Agriculture, Forestry and Fisheries	MAFF/PAFSC	Pharmaceutical Affairs Law
Canada	Canadian Veterinary Drugs Directorate	VDD	Food and Drugs Act (R.S.C., 1985, c. F-27); last amended on 2008-06-16

[a]Legislation and registration procedures for VMPs for therapeutic and prophylactic use and for feed additives are the same in some countries (Australia and USA) but separate in others (EU and Japan).
[b]FDA establishes safety guidelines for drug use in food-producing species and the US Department of Agriculture (USDA) enforces the standards set by FDA.
[c]In the EU, marketing authorizations may be granted either by EMA (centralized procedure) or national authorities (decentralized and mutual recognition procedures), but MRLs are set by EU.
[d]Formerly the European Medicines Evaluation Agency (EMEA). Note that some cited documents refer to EMEA.

authority, there are also national authorities; MAs can be obtained through four possible channels: centralized, decentralized, mutual recognition, and a solely national channel. For products containing AMDs, all authorities require the submission of data packages that establish their quality, safety, and efficacy (QSE). Considerable progress has been made in harmonizing QSE registration requirements in the form of guidelines, at international level under the auspices of VICH (International Co-operation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products; see Chapter 3).

2.5 DEFINITION, ASSESSMENT, CHARACTERIZATION, MANAGEMENT, AND COMMUNICATION OF RISK

2.5.1 Introduction and Summary of Regulatory Requirements

National and supranational regulatory authorities are responsible for the administration of legislation, designed to ensure that all foodstuffs obtained from animals are safe for human consumption. Safety in relation to human consumption of food containing (usually no more than trace amounts of) residues of drugs and their metabolites is based on a scientific assessment of data, which ultimately defines the risk. The risk, when defined, is used to establish a food-withholding period, which can be communicated to interested parties. Implementation of the withholding period is the responsibility of veterinarians, farmers, and others concerned with product use in clinical practice. In most countries, residue testing programs are now in place, to ensure as far as possible compliance with the statutory withholding periods. By these mechanisms, the general public is reassured that food derived from animals treated with drugs does not contain residues that might constitute a health hazard to consumers.

The health risks to human consumers of tissue residues of AMDs exceeding MRLs, or residues of AMDs for which no MRL has been determined, include direct toxicity to cells of the host, immunotoxicity (allergenicity), and the emergence of resistance in human GIT microflora and its subsequent spread. In addition, there is a requirement to achieve low concentrations of AMDs in milk to ensure non-interference with the manufacture of milk-derived products: cheese, butter, and yogurt. Concentrations of antimicrobials as low as 1 μg/kg can delay starter activity for these dairy products. Moreover, AMDs may decrease acidity and retard flavor production in butter manufacture, as well as inhibit the ripening of cheeses.

An important element of safety assessment comprises a series of studies designed to ensure that, when products are administered to food-producing species, a withholding period is established, which ensures that food from treated animals can be eaten safely by humans. Table 2.10 summarizes the range of extensive studies required to satisfy human food safety requirements. The process, which commences with basic pharmacokinetic studies, also includes metabolism studies and animal toxicity and microbiological studies (and in some instances pharmacological and immunotoxicity data are also required), designed to establish a series of NOELs (Table 2.11). VICH has issued harmonized guidelines for establishing the toxicity of VMPs (including AMDs) and also data requirements on antimicrobial properties relating to safety, as listed in Table 2.12.

TABLE 2.10 VICH Harmonized Guidelines on Various Toxicity Studies

Guideline No.	Year	Title
GL22	2001	Reproduction Toxicity Testing[a]
GL23	2001	Genotoxicity Testing[a]
GL27	2003	Pre-Approval Information for Registration of New Veterinary Medicinal Products for Food Producing Animals with Respect to Antimicrobial Resistance[b]
GL28	2002	Carcinogenicity Testing[a]
GL31	2002	Repeat-Dose (90 Day) Toxicity Testing[a]
GL32	2002	Developmental Toxicity Testing[a]
GL33	2004	General Approach to Testing[a]
GL36	2004	General Approach to Establish a Microbiological ADI[b]
GL37	2003	Repeat-Dose (Chronic) Toxicity Testing[a]

[a]Study evaluating the safety of residues of veterinary drugs in human food.

[b]Study evaluating antimicrobial potency and resistance.

Source: Guidelines accessed at `http://www.vichsec.org`

The standard battery of safety studies (Tables 2.10–2.12) is designed to establish the highest dose that produces no observed effect for non-carcinogenic substances. From the experimental data from all studies, the most sensitive effect in the species most predictive of humans is designated the toxicological NOEL. Allergenicity is not a significant issue for most AMDs. The main exception is benzylpenicillin. The evaluations conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the request of the Codex Alimentarius Commission (CAC) did not establish an ADI for benzylpenicillin, but recommended that the daily intake be kept below 30 μg of parent drug per day, setting MRLs of 0.05 mg/kg for edible tissues and 0.004 mg/kg for milk.[89]

The lowest NOEL (toxicological, pharmacological, or microbiological) is used to derive an *acceptable daily intake* (ADI), expressed in milligrams per person per day throughout life, through application of the following simple formula

$$\text{ADI} = \text{NOEL} \times \text{SF}$$

TABLE 2.11 No Observable Effect Levels: Definitions, Guidelines, and Applications

NOEL (and Guidelines)	Definition/Description	Application
Toxicological (VICH, GL22,23,28,31,32,33,37)	Determined in dose–response studies as the dose, in a battery of tests, which is the most sensitive toxicological effect in the species most predictive for humans	Used to determine the toxicological ADI from the relationship $ADI_{tox} = NOEL_{tox} \times SF$
Pharmacological (VICH, GL33)	Determined in dose–response studies, in a range of tests, as the dose that is the most sensitive pharmacological effect	For some drugs (e.g., glucocorticoids) a pharmacological action may be exerted at dose rate less than the toxicological NOEL; used to determine the pharmacological ADI from the relationship $ADI_{pharm} = NOEL_{pharm} \times SF$
Microbiological (VICH, GL27,36)	GL27 outlines the risk of transfer of resistant microorganisms or resistant determinants from animal foodstuffs to humans	—
	GL36 outlines the methods and test systems for determination of the microbiological NOEL	Required for all antimicrobial drugs to establish microbiological ADI
Immunotoxicological (VICH, GL33)	For some antimicrobial drug classes (e.g., β-lactams), immunotoxicity tests are required	Used to establish potential for eliciting allergic reactions in sensitive individuals

TABLE 2.12 Classification of Studies Required by Regulatory Authorities to Satisfy Human Food Safety Requirements on AMDs Residues

Study Type	Description and Objective
Toxicology studies *in vivo* in laboratory animals and *in vitro* studies for genotoxicity	For the range of VICH approved tests and guideline numbers, see Table 2.10; these studies establish a toxicological NOEL
In vitro studies to establish spectrum of activity and potency of AMDs	Establish a microbiological NOEL
Pharmacokinetic studies in laboratory animals and target species	Establish blood/plasma concentration–time profiles and derivation of key pharmacokinetic parameters and variables
Metabolism studies in laboratory animals and target species	Identify metabolites to determine whether a metabolite or parent compound is the marker residue
Residue depletion studies in target species	Define the rate of depletion of the marker residue in edible tissues and fluids (milk, honey), using the highest recommended dose; separate studies for each administration route
Validated analytical method	Identification and quantification of marker residues in animal tissues, milk, eggs, and honey by a determinative method; if the determinative method is not sufficiently specific, a confirmatory method for structural identification is required

where SF is a safety factor. SF may also be referred to as the *uncertainty factor* (UF), which perhaps reflects more accurately the intent of terminology, that is, the management of variability, but in this text we retain the traditional terminology. The ADI typically is based on an assumed body weight of 60 kg. In the case of a toxicological NOEL, SF usually has a value of 100 or higher (see Chapter 3). It is the product of two separate 10-fold factors that allows for interspecies difference and human variability. These 10-fold factors allow for both toxicokinetic and toxicodynamic differences and were subdivided to take into account each aspect separately.[90] Values of $10^{0.6}$ (i.e., 4) for toxicokinetics and $10^{0.4}$ (i.e., 2.5) for toxicodynamics are used for species differences, and equal values of $10^{0.5}$ (i.e., 3.16) for both toxocokinetics and toxicodynamics are used for human variability.[91,92] This value of 3 is not without experimental evidence; examining different databases, it was shown that multiplying a default SF by 3 allowed a coverage of 99% for an additional uncertainty factor.[93] In 2009, MacLachlan used physiologically based pharmacokinetic (PBPK) modeling (see Section 2.5.4.1) to explore for food-producing species the possible value of a default scaling factor based on physiological differences, which can be used to improve estimates of residues from lactating dairy cattle to other food-producing species.[94]

The safety of AMD residues must also be addressed with respect to the human intestinal flora, and derivation of a microbiological ADI is required by regulatory authorities, if AMD or microbiologically active residues reach the human colon. An appropriate ADI should prevent two

risks: a disruption of the colonization barrier and the possible increase of the population(s) of resistant bacteria. VICH GL 36 explains the required procedure.[6] The first step is to determine whether there is a need for a microbiological ADI. Data to assess whether a microbiological ADI is needed may be obtained experimentally or from the literature. If required, disruption of the colonization barrier and possible change of resistant bacteria should be documented. Microbiological data may be generated in humans *in vivo*, in gnotobiotic animals, or *in vitro* in species and strains of bacteria accepted as representative of the human GIT flora. Currently, the VICH guideline does not recommend any particular tests, because the reliability and validity of currently used *in vitro* and *in vivo* test systems is not fully established. Finally, the ADI is derived either from *in vitro* data, taking into account a non-observable adverse effect concentration (NOAECs) or from *in vivo* data from a NOEL divided by an uncertainty factor. Within most jurisdictions there is also a requirement to quantify the potential effect of the AMD on starter cultures used in food processing (cheese, buttermilk, sour cream, yogurt starter cultures, etc.).

The lowest ADI (usually toxicological or microbiological) is defined as the amount of drug plus metabolite residue that can be consumed daily for a lifetime, without appreciable risk to the health of the consumer. However, some residues can give rise to an acute rather than a chronic toxicological effect. β-Agonists are an example; they can induce shortlived pharmacological responses, such as tachycardia, but with no long-term consequences. Some authorities have accepted, for such drugs, an acute reference dose (ARfD) as the appropriate health standard.

On the basis of the lowest ADI (usually toxicological or microbiological), together with metabolism and residue depletion studies, the MRL of the residues is determined for each tissue, expressed in μg/kg on a freshweight basis. The range of extensive studies involved in setting ADIs is summarized in Tables 2.10 and 2.12.

Metabolism studies are required in the laboratory animal species used to determine the toxicological NOEL, as well as each food-producing animal species. ADIs are based on total residues of drug plus all metabolites, whereas MRLs comprise a single, quantifiable marker residue, most commonly the parent compound but in some instances a single metabolite or a mixture of compounds. To establish the MRL for each tissue, food consumption estimates are made on the basis of an assumed standard meal (the so-called food basket), as discussed further in Section 2.5.2.2.

The composition of the standard meal varies between regulatory authorities, so that MRLs and WhTs also differ, even when the ADIs are the same. The differences in MRLs adopted by national bodies are attributable mainly to the levels of risk each is prepared to accept, the conditions of use, and methods for establishing MRLs. These differences in national standards affect international trade in animal foods adversely, as manufacturers are required to comply with diverse standards imposed by several importing countries. The MRLs for veterinary drugs developed by the Codex Alimentarius Commission (CAC) are designed to protect the consumer, to be compatible with good veterinary practice in drug use, and to facilitate fair practices in international trade. These are objectives of the Codex Committee on Veterinary Drug Residues in Foods (CCVDRF).

Although CAC MRLs have been adopted by many countries, they are not mandatory.

MRLs are published at the following sites:

United States: `http://www.fsis.usda.gov/OPHS/red_book_2001/2001_Residue_Limits_Veterinary_Drugs_App4.pdf`

Canada: `http://www.hc-sc.gc.ca/dhp-mps/vet/mrl-lmr/mrl-lmr_versus_new-nouveau_ehtml`

EU: `http://ec.europa.eu/health/files/mrl/mrl_20101212_consol.pdf`. EU MRL Summary reports are located at `http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/vet_mrl_search.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058008d7ad`.

The CCRVDF has a primary function in establishing internationally acceptable concentrations of veterinary drugs and their metabolites in food animal products. It thereby facilitates world trade in agricultural products by establishing internationally accepted standards, recommendations, codes of practice, and guidelines, based on a consensus of expert scientific opinion. Space here does not allow provision of a full list of MRLs for all regulatory authorities, but Table 2.13 summarizes marker residues, target tissues, and approved MRLs for AMDs, together with qualifying provisions, where appropriate, adopted in the EU. The MRL for each compound is contained in the European Public MRL Assessment Reports (EPMARs), published on the European Medicines Authority website. Table 2.14 lists tolerances of selected AMDs in the United States, and, for comparative purposes, the ratios of these tolerances to MRLs adopted by the EU are presented. The data illustrate both similarities and differences between the two jurisdictions.

A risk analysis framework for food safety has been developed as an approach to assessing the relationship between potential hazards and the actual human health risks.[95] The three components of risk analysis are assessment, management, and communication.

2.5.2 Risk Assessment

Risk assessment, where risk is the probability of harm to the consumer, is represented by the relationship risk = hazard × exposure. IPCS defined these terms as follows:[96]

TABLE 2.13 Marker Residues and Target Tissues for Antimicrobial Drugs for Which MRLs Have Been Defined in the EU

Pharmacological Class	Individual Drugs	Marker Residue (Par = Parent Drug)	Animal Species[a]	Target Tissues[b] and MRL (μg/kg)	Other Provisions
Sulfonamides	All sulfonamides	Par	All	M, F, L, K, Mi (all 100)	Combined total residues of all sulfonamides not >100 μg/kg
			B, C, O	Mi100	
Diaminopyrimidines	Baquiloprim	Par	B	F10, L300, K150, Mi(30)	—
			P	F40, L50, K50	—
	Trimethoprim	Par	All except E	M, F, L, K, Mi (all 50)	Not eggs[c]
			E	M, F, L, K (all 100)	—
Penicillins	Amoxicillin	Par	All	M50, F50, L50, K50, Mi 4	—
	Ampicillin	Par	All	M50, F50, L50, K50, Mi 4	—
	Benzylpenicillin (penicillin G)	Par	All	M50, F50, L50, K50, Mi 4	—
	Cloxacillin	Par	All	M300, F300, L300, K300, Mi 30	—
	Dicloxacillin	Par	All	M300, F300, L300, K300, Mi 30	—
	Nafcillin	Par	All ruminants	M300, F300, L300, K300, Mi3	Intramammary use only
	Oxacillin	Par	All	M300, F300, L300, K300, Mi30	—
	Penethamate	Benzylpenicillin	All mammalian food species	M50, F50, L50, K50, Mi4	—
	Phenoxymethyl penicillin	Par	P	M, L, K (all 25)	—
			Po	M, F, L, K (all 25)	—
Cephalosporins	Cefacetrile	Par	B	Mi125	Intramammary use only
	Cefalexin	Par	B	M200, F200, L200, K1000, Mi100	—
	Cefalonium	Par	B	Mi20	—
	Cefapirin	Sum of cephapirin + desacetylcephapirin	B	M50, F50, K100, Mi60	—
	Cefazolin	Par	B, O, C	Mi50	—
	Cefoperazone	Par	B	Mi50	—
	Cefquinome	Par	B	M50, F50, L100, K200, Mi20	—
			P	M50, F50, L100, K200	—
			E	M50, F50, L100, K200	—
	Ceftiofur	Sum of all residues retaining the β-lactam structure expressed as desfuroylceftiofur	All mammalian food-producing species	M1000, F2000, L2000, K6000, Mi100	—
Quinolones	Danofloxacin	Par	All food-producing species except B, O, C, P Po	M100, F50, L200, K200	—
			B, O, C	M200, F100, L400, K400	—
			Po	M200, F100, L400, K400	Not eggs[c]

(*continued*)

TABLE 2.13 (*Continued*)

Pharmacological Class	Individual Drugs	Marker Residue (Par = Parent Drug)	Animal Species[a]	Target Tissues[b] and MRL (μg/kg)	Other Provisions
	Difloxacin	Par	All food-producing species except B, O, C, Po	M300, F100, L800, K600	—
			B, O, C	M400, F100, L1400, K800	—
			P	M400, F100, L800, K800	—
			Po	M300, F400, L1900, K600	Not eggs[c]
	Enrofloxacin	Sum of enrofloxacin and ciprofloxacin	All except B, O, C, P, Po, R	M100, F100, L200, K200	—
			B, O, C	M100, F100, L300, K200, Mi100	—
			P, R	M100, F100, L200, K300	—
			Po	M100, F100, L200, K300	Not eggs[c]
	Flumequine	Par	All except B, O, C, P, Po, Fi	M200, F250, L500, K1000	—
			B, P, O, C	M200, F300, L500, K1500, Mi50	—
			Po	M400, F250, L800, K1000	—
			Fi	M600	—
	Marbofloxacin	Par	B	M150, F50, L150, K150, Mi75	—
			P	M150, F50, L150, K150	—
	Oxolinic acid	Par	All except Fi	M100, F50, L150, K150	Not eggs or milk[c]
			Fi	M100	—
	Sarafloxacin	Par	Ch	F10, L100	—
			S	M30	—
Macrolides	Erythromycin	Erythromycin A	All	M200, F200, L200, K200, Mi40, Eg150	—
	Spiramycin	Sum of spiramycin + neospiramycin	B	M200, F300, L300, K300, Mi 200	—
			Ch	M200, F300, L400	—
		Spiramycin 1	P	M250, L2000, K1000	—
	Tilmicosin	Par	All except Po	M50, F50, L1000, K1000, Mi50	—
			Po	M75, F75, L1000, K250	—
	Tulathromycin	(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-ethyl-3,4,10,13-tetra-hydroxyl-3,5,8,10,12,14-Hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopent-decan-15-one expressed as tulathromycin equivalents	B, P	F100, L3000, K3000	Not milk[c]
	Tylosin	Tylosin A	All	M100, F100, L100, K100, Mi50, Eg200	—

TABLE 2.13 *(Continued)*

Pharmacological Class	Individual Drugs	Marker Residue (Par = Parent Drug)	Animal Species[a]	Target Tissues[b] and MRL (μg/kg)	Other Provisions
	Tylvalosin	Sum of tylvalosin + 3-*o*-acetyltylosin	P	M50, F50, L50, K50	—
			Po	F50, L50	Not eggs[c]
Phenicols	Thiamphenicol	Par	All	M50, F50, L50, K50, Mi50	Not eggs[c]
	Florfenicol	Sum of florfenicol and its metabolites measured as florfenicol amine	B	M200, L3000, K300	—
Tetracyclines	Chlortetracycline	Sum of par and its 4-epimer	All	M100, L300, K600, Mi100, Eg200	—
	Doxycyline	Par	B	M100, L300, K600	Not milk[c]
			P	M100, F300, L300, K600	—
			Po	M100, F300, L300, K600	Not eggs[c]
	Oxytetracycline	Sum of par & its 4-epimer	All	M100, L300, K600, Mi100, Eg200	—
	Tetracycline	Sum of par & its 4-epimer	All	M100, L300, K600, Mi100, Eg200	—
Naphthalene-ringed ansamycin	Rifaximin	Par	B	Mi60	—
Pleuromutilins	Tiamulin	Sum of metabolites that may be hydrolysed to 8-a-hydroxymutilin	P, R	M100, L500	—
			Ch	M100, F100, L1000	—
			T	M100, F100, L300, Eg1000	—
	Valnemulin	Par	P	M50, L500, K100	—
Lincosamides	Lincomycin	Par	All	M100, F50, L500, K1500, Mi150, Eg50	—
	Pirlimycin	Par	B	M100, F100, L1000, K400, Mi100	—
			P	M100, F50, L500, K1500	—
			Ch	M100, F50, L500, K1500, Eg50	—
Aminoglycosides	Apramycin	Par	B	M1000, F1000, L10,000, K20,000	Not milk[c]
	Dihydrostreptomycin	Par	All ruminants	M500, F500, L500, K1000, Mi200	—
			P, R	M500, F500, L500, K1000	—
	Gentamicin	Sum of gentamicin C_1 gentamicin C_{1a}, gentamicin C_2 + gentamicin C_{2a}	B	M50, F50, L200, K750, Mi100	—
			P	M50, F50, L200, K750	—
	Kanamycin	Kanamycin A	All except Fi	M100, F100, L600, K2500, Mi150	—
	Neomycin (including framomycin)	Neomycin B	All	M500, F500, L500, K5000, Mi1500, Eg500	—
	Paromomycin	Par	All	M500, L1500, K1500	Not eggs or milk[c]
	Spectinomycin	Par	All except O	M300, F500, L1000, K5000, Mi200	Not eggs[c]
			O	M300, F500, L2000, K5000, Mi200	—

(continued)

TABLE 2.13 (*Continued*)

Pharmacological Class	Individual Drugs	Marker Residue (Par = Parent Drug)	Animal Species[a]	Target Tissues[b] and MRL (μg/kg)	Other Provisions
	Streptomycin	Par	All ruminants	M500, F500, L500, K1000, Mi200	—
			P, R	M500, F500, L500, K1000	—
Polypeptides	Bacitracin	Sum of bacitracin A, bacitracin B, + bacitracin C	B	Mi100	—
			R	M150, F150, L150, K150	—
β-Lactamase inhibitors	Clavulanic acid	Par	B	M100, F100, L200, K400, Mi200	—
			P	M100, F100, L200, K400	—
Polymyxins	Colistin	Par	All	M150, F150, L150, K200, Mi50, Eg300	—
Orthosamomycins	Avilamycin	Dichloroisoeverninic acid	P, Po, R	M50, F100, L300, K200	Not eggs[3]
Ionophores	Monensin	Monensin A	B	M2, F10, L30, K2, Mi2	—
	Lasalocid	Lasalocid A	Po	M20, F100, L100, K50, Eg150	—
Miscellaneous	Novobiocin	Par	B	Mi50	—

[a]Abbreviations used in this column: all = all food-producing species; B = bovine; C = caprine; Ch = chicken; E = equidae; Fi = fin fish; O = ovine; P = porcine; Po = poultry; R = rabbit; S = salmonidae; T = turkey.
[b]Abbreviations used in this column: M = muscle; F = fat; L = liver; K = kidney; Mi = milk; Eg = eggs (for poultry, chickens, and pigs, F = skin and fat; for fin fish and salmonidae, M = muscle and skin in natural proportions).
[c]Not for use in animals from which eggs and/or milk are produced for human consumption.
Source: Data from EU Commission Regulation 37/2010.[102]

Hazard is the inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub) population is exposed.

Risk is the probability of an adverse effect in an organism, system, or (sub) population caused under specified circumstances by exposure to an agent.

2.5.2.1 *Hazard Assessment*

For AMD residues in foodstuffs, the hazard is drug/drug metabolite residues and exposure is dietary intake.[97] As risk assessment must be applied independently to each AMD, it follows that the database identifying hazard must be extensive and should comprise, for each drug, information on structure, purity, physicochemical, pharmacological (including pharmacokinetic and metabolism data), and toxicological properties. Data may be obtained from human epidemiological and animal toxicology studies and *in vitro* assays (e.g., for mutagenicity/genotoxicity).

After hazard identification, hazard characterisation is undertaken, and this is normally based on dose–response relationships in the range of toxicological studies summarized in Section 2.5.1. It is assumed that a threshold dose for response can be identified, where the NOEL is the highest dose that causes no (adverse) detectable effect in the most sensitive animal species or strain. Other approaches have been used, however, such as determination of a benchmark dose (BMD).[98] Determination of BMD involves modeling of all dose–response data, with increased weighting applied to data at low levels of response (e.g., ED_{05}, ED_{10}, doses producing respectively 5% or 10% of maximum), with the subsequent application of safety/uncertainty factors in determining ADI. As discussed in Section 2.5.1, the ADI is likely to differ between regulatory authorities.

The NOEL procedure for determining ADI is not acceptable for drugs with effects that are characterized by non-threshold mechanisms. An example is genotoxic carcinogens, which cause genetic alterations in target cells. Mutagenicity tests are carried out to provide evidence of genotoxicity. Such compounds are assumed to be harmful at any exposure and are banned from use in food-producing animals in many countries. Other countries accept their use, provided residue concentrations are small enough to be regarded as posing negligible risk. On the other hand, non-genotoxic carcinogens act extragenetically to cause enhanced cell proliferation or sustained hyperfunction, dysfunction, or both. At least theoretically, non-genotoxic carcinogens can be regulated through the NOAEL determined ADI procedure.

A final step in hazard characterization leading to setting the ADI is for the regulatory authority to reflect on the significance and applicability of those responses revealed in high-dose-rate toxicology studies to the circumstance of

TABLE 2.14 FDA Tolerance Levels for Some AMD Residues in Edible Animal Meat Products and EU MRL: USA Tolerance

Compound	Animal Species, USA	Tolerance, USA (μg/kg)	Ratio EU MRL: USA Tolerance
Amoxicillin	Bovine	10 (M, K, L, F)[a]	5 : 1 all tissues
Ampicillin	Bovine, porcine	10 (M, K, L, F)	5 : 1 all tissues
Benzylpenicillin	Bovine	50 (M, K, L, F)	1 : 1 all tissues
	Turkey	10 (M, K, L, F)	5 : 1 all tissues
Ceftiofur	Bovine	1000 (M), 2000 (L), 8000 (K)	M1 : 1, L1 : 1, K0.75 : 1
Cephapirin	Bovine	100 (M, K, L, F)	0.5 : 1
Cloxacillin	Bovine	10 (M, K, L, F)	30 : 1
Danofloxacin	Bovine	200 (M, L)	M1 : 1, L2 : 1
Dihydrostreptomycin	Bovine, porcine	2000 (K), 500 (M, L, F)	M, L, F 1 : 1, K 0.5 : 1,
Enrofloxacin	Bovine	100 (L)	L 3 : 1
	Chicken, turkey	300(M)	M 0.33 : 1
Erythromycin	Bovine, porcine	100 (M, K, L, F)	2 : 1 all tissues
	Chicken, turkey	125 (M, K, L, F)	1.6 : 1 all tissues
Florfenicol	Bovine	300 (M), 3700 (L)	M 0.66 : 1, L0.8 : 1
	Porcine	200 (M), 2500 (L)	M 0.66 : 1, L0.8 : 1
Gentamicin	Porcine	100 (M), 300 (L), 400 (K, F)	M 0.5 : 1, L0.66 : 1, K 1.88 : 1, F 0.125 : 1
Lincomycin	Porcine	100 (M), 600 (L)	M 1 : 1, L 0.83 : 1
Neomycin	Bovine, ovine, porcine	1200 (M), 3600 (L), 7200 (K)	M 0.42 : 1, L 0.14 : 1, K0.69 : 1
Pirlimycin	Bovine	300 (M), 500 (L)	M 0.33 : 1, L 2 : 1
Spectinomycin	Bovine	250 (M), 4000 (K)	M 1.2 : 1, K 1.25 : 1
	Chicken, turkey	100 (M, K, L, F)	M 3 : 1, K50 : 1, L20 : 1, F5 : 1
Streptomycin	Bovine, porcine	500 (M, L, F), 2000 (K)	M, L, F 1 : 1, K 0.5 : 1
Sulfonamide group	Bovine, porcine, poultry	100 (M, L. K, F)	1 : 1 all tissues
Tiamulin	Porcine	600 (L)	L 0.83 : 1
Tilmicosin	Bovine, ovine	100 (M), 1200 (L)	M 0.5 : 1, L 0.83 : 1
	Porcine	100 (M), 7500 (L)	M 0.5 : 1, L0.13 : 1
Tylosin	Bovine, porcine, chicken, turkey	200 (M, L, K, F)	0.5 : 1 all tissues
Tetracycline group	Bovine, ovine, porcine, poultry	2000 (M), 6000 (L), 12000 (F, K)	M, L, K 0.05 : 1, F 0.025 : 1

[a]Tissue: F, fat; L, liver; K, kidney; M, meat

Source: Adapted from Croubels et al. (2004).[35]

residue intake, when the latter may be several orders of magnitude less than the former. Further information on the process by which an ADI is typically assigned for an AMD is provided in Chapter 3.

2.5.2.2 *Exposure Assessment*

Three factors determine exposure assessment: quantity of food consumed, residue concentration in that food, and the marker residue : total residue ratio. The "food basket" adopted by most authorities comprises:

- 300 g muscle (for fish, muscle and skin in natural proportions).
- 50 g fat (for pigs and poultry, fat and skin in natural proportions, for poultry 90 g)
- 100 g liver
- 50 g kidney (10 g poultry)
- 100 g eggs
- 20 g honey
- 1.5 l milk

In the EU, for example, the food basket comprises, for mammals, muscle (300 g), liver (100 g), kidney (50 g), and fat (50 g) and, if appropriate, milk, eggs, and honey (for poultry, 10 g kidney and 90 g fat). For poultry and pigs, the MRL for fat relates to fat and skin in natural proportions in the EU, while for fin fish, muscle includes muscle and skin in natural proportions. In general, a numerically greater MRL is allowed for foods likely to be consumed infrequently or in small amounts (e.g., kidney relative to muscle). In addition, residues that occur in food of plant origin or that come from the environment need to be considered when fixing the MRL.

On the basis of the food basket, the EU authority (EMA/CVMP) then requires applicants for MAs to estimate the theoretical maximum daily intake (TMDI) for persons weighing 60 kg, applying the equation:

$$\text{TMDI} = \sum \left(\text{daily intake}_i \times \text{MRL}_i \times \frac{\text{TR}_i}{\text{MR}_i} \right)$$

where daily intake$_i$ (kg) = daily consumption as defined in the model food basket; MRL$_i$ = MRL (μg/kg) for muscle,

fat, liver, kidney, eggs, and honey; TR_i = total residue concentration (or pharmacological or microbiological activity where relevant); and MR_i = marker residue concentration (or pharmacological or microbiological activity where relevant) in the same tissues and commodities.

Later, JECFA proposed an alternative to TMDI, namely the estimated dietary intake (EDI),[99] which has been accepted by the Australian authority. The difference from TMDI is the replacement of MRL by median residue concentration, on the reasonable consideration that, in the chronic intake circumstance, MRL does not provide a realistic estimate of residue intake. MRL is the upper limit of a high percentile (usually 95th) of the distribution of marker residue. In contrast, the median residue concentration provides the best point estimate of the central tendency over a prolonged period.

For a 60-kg person, EDI is calculated from the equation

$$\text{EDI} = \sum \left(\text{daily intake}_i \times \text{median residue concentration}_i \times \text{MRL}_i \times \frac{\text{TR}_i}{\text{MR}_i} \right)$$

where daily intake_i (kg) = daily consumption as defined in the model food basket; median $\text{residue concentration}_i$ = median residue concentration (μg/kg) for muscle, fat, liver, kidney, eggs, and honey; TR_i = total residue concentration; and MR_i = marker residue concentration in the same tissues and commodities.

In the United States, FDA/CVM reasonably assumes that an individual consuming 300 g of muscle will not, on a given day, also consume liver or kidney but might well consume a full allocation of milk and eggs. FDA therefore calculates a safe concentration of total residues for edible tissues (and if appropriate additionally milk and eggs) from the equation:

$$\text{SC} = \frac{\text{ADI} \times 60 \text{ kg}}{\text{FCF}}$$

where SC = safe concentration for total residues in a specified edible tissue, as defined in the model food basket; ADI = acceptable daily intake; and FCF = daily consumption of the specified edible tissue.

It is clear that all regulatory authority approaches to predicting dietary exposure are very conservative, in that all are higher than actual dietary intake. These conservative assumptions are as follows: daily consumption for a lifetime of the model food basket, the treatment of all animals at the maximum recommended dose rate and duration, slaughter of treated animals at the WhT, and the presence of residues in all edible tissues (including milk and eggs) at the MRL (TMDI calculation) or at median residue concentrations (EDI calculation). The conservative assumptions used to calculate exposure are additional to the conservative assumptions made in the toxicology studies used to determine NOELs.

2.5.3 Risk Characterization

The characterization of risk involves consideration of the information garnered when identifying and characterizing the hazard together with exposure assessment. For AMDs used in food-producing species, the outcome comprises the MRLs derived from the ADI approach together with application of the model meal.[100] In characterizing risk, two extreme examples may be cited. On one hand, residues can constitute a health hazard at any concentration, so that MRLs cannot be established and use of the AMD in food-producing animals is disallowed. On the other hand, drugs may leave residues that are not considered to pose a health risk to humans, and MRLs are therefore not required. The latter applies to many excipients used in VMPs. Most AMDs lie between the two extremes cited, but furans that are both mutagenic and carcinogenetic are banned from use in food-producing animals. Similarly, nitroimidazoles (metronidazole, ronidazole) are suspected to be mutagens and carcinogens, and their use in food-producing animals is prohibited. In the EU, under Council Regulation (EEC) 2377/90[44] and up until the entry in force of a new regulation EC 470/2009[101] laying down European Community (EC) procedure for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, all pharmacologically active substances were until recently included in one of the following annexes:

- Annex I—definitive MRLs have been set.
- Annex II—MRLs are not required for the protection of public health.
- Annex III—provisional MRLs have been set, but finalization has not been completed.
- Annex IV—substances are prohibited from use in VMPs for all food-producing animals.

European Community Regulations 470/2009[101] and 37/2010[102] have introduced a new system of classification, whereby all pharmacologically active substances are now listed in a single annex in alphabetical order in two tables, the first to include all compounds listed in Annexes I, II, and III and a second table listing prohibited substances from Annex IV. In the United States procedures are broadly similar [see *Code of Federal Regulations* (CFR) Title 21, Part 556, on the FDA website[103]]. In the United States a tolerance is not required if there is no reasonable expectation that residues may be present, or when the drug is metabolized or assimilated into tissues in such form

that any possible residue would be indistinguishable from normal tissue constituents.

In summary, to establish MRLs for a given drug requires provision of the following data: knowledge of dosage schedule (amount, dose interval, duration) and administration route; pharmacokinetic and metabolic information in laboratory animals and each of the target food-producing species; residue depletion in each target species using radiolabeled drug; validated analytical methods for detection and quantification of residues, including marker residue; and data defining the effect of residues on food processing. It is not possible to address fully all of these aspects here, but the reader is referred to (1) Section 2.3 of this chapter on AMD pharmacokinetics and metabolism; (2) later chapters of this text and MacNeil,[104] describing analytical method requirements and validation; and (3) an excellent account in 2010 on risk characterization in relation to residues provided by Reeves.[105]

There is no consistency in procedures used to establish MRLs either between national authorities and JECFA or, indeed, between different national authorities. This is regrettable from a sponsor's perspective, as it may involve, at worst, several similar studies (with major implications for animal welfare and added expense) and variance in achieving (or not) a MA between jurisdictions. The rational procedures proposed by JECFA for MRL determination are based on three premises: (1) they can be enforced by regulatory programs that use available analytical methods, (2) they are no higher than necessary, and (3) they reflect residue concentrations expected when the product containing AMD is used in accordance with good veterinary practice. For this latter point (3), JECFA introduced the concept of the so-called estimated daily intake (EDI). They soundly assumed that EDI is a more appropriate tool in determining whether residues pose any chronic (lifetime) risks than does TDMI. In other words, the JECFA approach copes only with chronic, not acute, exposure that is a matter of concern for CVMP.[106]

The new JECFA pivotal approach to determine a MRL is to use median residue concentrations (not MRL) in animal-derived food for the calculation of the EDI from a model daily food basket. JECFA combines an iterative approach with a statistical tool, as agreed to at the 66th JECFA meeting.[107] A linear regression analysis is performed on the terminal depletion of marker residue in edible tissues after the last administration of drug. If appropriate, the data from the analysis enable calculation of the upper limits of the 95% confidence interval of the upper one-sided tolerance limit of the 95th percentile of the test animal. Then the JECFA approach defines a link between daily residue intake as expressed through the EDI and MRL as follows:

> The MRL and the median concentration are derived from the same time point of the depletion data of the marker residue. The MRL is a point on the curve describing the upper one-sided 95% confidence limit over the 95th percentile. The median is the corresponding point on the regression line for the same time point. Both figures are obtained from a statistical evaluation of the data.

Practically, the MRL is that point on the tolerance limit as defined above at or beyond which the predicted EDI using the median concentration guarantees that EDI$\leq$ADI.

In contrast, the EU approach consists of computing a TMDI using MRL, not median residue concentrations; CVMP criticized the JECFA approach for its intrinsic limitation to a chronic risk scenario and also for several technical reasons, including difficulties of using linear regression to estimate the tolerance limit of residue concentrations with its confidence interval and to the rather loose concept of good veterinary practice. (For further details, see Ref. 106. For further details on and a discussion of the USFDA approach, see Refs. 105 and 108.)

2.5.4 Risk Management

2.5.4.1 Withholding Times

In relation to the use of AMDs in food-producing species, risk is managed by setting withdrawal/withholding periods, defined as the time interval from last administration of a product to when the animal can be slaughtered to provide animal foods, milk, eggs, or honey that can be safely consumed. WhTs are set by regulatory authorities. Adherence to the WhT provides assurance that food derived from treated animals will not exceed the MRL for the drug substance. The normal procedure for assigning the WhT is to utilize the data generated in residue depletion studies conducted: (1) with non-radiolabeled drug; (2) in the product formulation proposed for marketing; and (3) administered at the highest dose rate, shortest dose interval, and longest duration specified in the product literature. Typically, a company would use a minimum of four animals of the target species at each of at least four slaughter times to define the depletion profile. The animals should be typical of target animals in clinical use, for example, young calves *or* lactating adult cattle. Extensive tables of tissue depletion pharmacokinetic data have been published by Craigmill et al.[109]

A simple approach is used in Europe when no statistical approach is possible; it consists of setting the WhT as the time when residues in all tissues from all investigated animals have been depleted to less than their respective MRLs and, to add to the observed delay, an additional, arbitrary safety span to compensate for the uncertainties of biological origin. This is the so-called pragmatic EU approach. The size of the safety span (typically 10–30%) was documented by comparing the WhT obtained by the regular EU statistical method for 62 depletion studies and the WhT that would

have been fixed using the so-called pragmatic approach; it was shown that the median value of the appropriate safety span was 25% but the range was from −24% to 233%, indicating that for some studies, selecting an arbitrary safety span of 10–30% is not conservative enough.[110]

The second and preferred method is to apply appropriate statistical analysis to the dataset, based on linear regression. Both EU and USFDA authorities assume log-linear decline of residue concentrations and apply least-squares regression to derive the fitted depletion line. Then the one-sided upper tolerance limit (95% in EU and 99% in USA) with a 95% confidence level is computed. The WhT is the time when this upper one-sided 95% tolerance limit for the residue is below the MRL with 95% confidence. In other words, this definition of the WhT says that at least 95% of the population in EU (or 99% in USA) is covered in an average of 95% of cases. It should be stressed that the nominal statistical risk that is fixed by regulatory authorities should be viewed as a statistical protection of farmers who actually observe the WhT and not a supplementary safety factor to protect the consumer even if consumers indirectly benefit from this rather conservative statistical approach.

Concordet and Toutain initiated a scientific debate on the estimation of WhTs, using a regression method to estimate a 99th (USA) or a 95th (EU) percentile of the population with a 95% confidence level.[111,112] The regression approach requires a pharmacokinetic/residue study, then modeling the depletion curve as a straight line after logarithmic transformation. Five assumptions are involved in applying linear regression:

1. No experimental uncertainty on the time of slaughter (x axis)
2. Linearity of the depletion curve
3. Normality of distribution of the logarithm of residue concentrations at each slaughter time
4. Homoscedasticity, that is, assumption of constant variance
5. Independence of observation (as outlined in detail by Concordet and Toutain[112]), which may or may not be satisfied in every instance.

As an alternative to the regression method, Concordet and Toutain[111,112] proposed a simple, understandable non-parametric method that allows the control of risk, defined as a percentage less than $100/1 - \alpha$. The only assumption is that the probability of exceeding MRL decreases monotonically with time. The method requires a few assumptions, including assuming that observations are independent and slaughter times are chosen during the declining phase of residue kinetics. The latter condition is especially important with controlled, slow-release formulations. For details of the method, see Concordet and Toutain.[111] They emphasize the advantage that all animals/samples contribute to WhT estimation, including those for which concentrations are lower limit of quantification (LLOQ) of the analytical method. The limit of this approach is its low statistical power requiring many more animals than the conventional regression method.

Martinez et al.[113] compared USFDA approaches on WhTs with those of Concordet and Toutain[111,112] (see Table 4 of Martinez et al.[113]) and concluded that the USFDA regression method was, on several grounds, more appropriate for prevention of exposure to non-compliant residues. The approach of EMA/CVMP is similar to that of USFDA, except that it is based on 95/95% tolerance limits, considered preferable because of uncertainty in extrapolating to extreme percentages of the population. Fisch extended the discussion on estimating WhTs.[114] He proposed the application of Bayesian methods, using Markov chain Monte Carlo methods for circumstances in which neither regression nor non-parametric approaches apply. In an early review, Martin-Jimenez and Riviere indicated a possible role for and relevance of using population pharmacokinetic data for describing drug disposition in fluids and drug deposition as residues in edible tissues.[115] However, they pointed out that adequate strategies were not available then, and indeed, this remains the case. Nevertheless, they highlighted the prospect and value at some future time of using multicompartment models to characterize plasma–tissue relationships. They even envisaged the possibility of defining WhTs with appropriate confidence intervals for subpopulations, depending on differences in clinical or production parameters. Such models would use a Bayesian approach to harvest information from a range of protocols (efficacy, safety, residues) pooled in a single model.

The most promising research in the area of residues and WhT is that of physiologically based pharmacokinetic modeling (PBPK) as illustrated for oxytetracycline in sheep.[116] Another example is the prediction of sulfonamide concentrations in pigs for prediction of non-compliant residues.[117] The principle of PBPK modeling is to develop a model with a generally complicated structure, including explicitly critical anatomical (e.g., polygastric vs. monogastric stomach), physiological, physicochemical, and biochemical processes regarding the purpose for which the model is developed. For example, a PBPK model intended to explore the consequence of inhibition of intestinal enzymes on WhT in milk in a given species would include explicitly not only the different compartments representing the relevant tissue for food safety (muscle, adipose tissue, kidney, liver, other carcass tissues, and also milk) but also a clearance component at the intestinal level. PBPK models should be viewed as sophisticated dosimetry models that offer great flexibility in modeling exposure scenarios for which there are no or limited data in order to predict tissue concentrations between varying routes of exposure and across species. MacLachlan

developed such a model in lactating cattle to explore the influence of differing physiological statuses on residues of lipophilic xenobiotics in livestock.[94] Interspecies extrapolation is a key issue for the establishment of WhT in minor species. The application of a PBPK model to the prediction of WhTs for oxytetracycline in salmon has demonstrated the applicability of PBPK modeling to the prediction of tissue residues in food animals and the establishment of WhTs.[118] More recently, it was shown that a PBPK model developed for midazolam in the chicken and then adapted to take into account species-specific physiological parameters for turkey, pheasant, and quail provided good predictions of the observed tissue residues in each species, in particular for liver and kidney.[118,119]

There are differences between regulatory authorities in procedures used to set milk withholding periods. USFDA/CVM requires use of at least 20 animals and analysis of milk samples for the marker residue in triplicate.[120] If the product is authorized for mastitis treatment, it is assumed that no more than one-third of the milk is derived from treated cows. A regression line is fitted to the log residue concentration data for each cow, and then fitted lines are used to estimate the distribution of log residue concentrations at each sampling time. Between-animal variance and measurement error variability are estimated and used to calculate a tolerance limit at each time. The WhT is set as the first time at which the upper 95% confidence limit of the 99th percentile of residue concentrations is equal to or less than the MRL.

For milk, EMA/CVMP uses a "time to safe concentration" (TTSC) approach.[121] TTSC is the first time when the upper 95% confidence limit of the 95th percentile of individual milk sampling times complies with the MRL. The method assumes a log normal distribution of individual times to safe concentration. If the data set is not suitable for analysis by the TTSC method, alternative statistical approaches may be used. Thus, the distributional assumptions of the USFDA/CVM and EMA/CVMP relate, respectively, to residue concentrations and time to safe concentration. An advantage of the latter approach is that an assumption of log linear depletion of residues is not made.

2.5.4.2 *Prediction of Withdholding Times from Plasma Pharmacokinetic Data*

Gehring et al.[122] and Riviere and Sundlof[123] have proposed that an approximation to WhT can be derived from the equation:

$$\mathrm{WhT} = 1.44 \times \ln\left(\frac{C_0}{\mathrm{MRL}}\right) \times T_{1/2}$$

where C_0 = concentration of drug in target tissue at the end of administration and $T_{1/2}$ is terminal half-life. While this equation is an approximation, it does provide a perspective on WhT relative to terminal half-life. If the marker residue is a metabolite, it is the terminal half-life of metabolite that should be used to estimate WhT. Assuming homogeneous drug distribution in the body (admittedly a tall order!), a therapeutic AMD plasma concentration of 10 mg/l and a MRL of 0.01 mg/l, then

$$\mathrm{WhT} = 1.44 \times \ln(10/0.01) \times T_{1/2} = 9.947 \times T_{1/2}$$

Thus, a WhT would be very slightly less than 10 half-lives if the ratio C_0/MRL were 1000. This "rule of 10" is based on the elimination of 99.9% of drug after 10 half-lives. If MRL is low relative to C_0, this will lead to a greater WhT value. If $T_{1/2}$ is short (e.g., for penicillin), WhT is correspondingly short. However, if tissue half-life is long (as for aminoglycosides), WhT can be very protracted. If drug dose is doubled, WhT is increased by a single half-life to 10.94 half-lives, showing that an error on the dose exerts a rather limited influence (of approximately 10%) on WhT. However, there is a circumstance in which the increase in dose increases a WhT disproportionally. This occurs when the residue depletion curve obeys a multiexponential decay and when the MRL value transects a phase having a relatively short half-life, when the dose is low but a very late terminal phase, when the dose is increased or when multiple drug administrations lead to some "stacking" as reported by KuKanich et al. for a long-acting formulation of florfenicol.[124] This can result in illegal residues when the product is administered for more than the single-label dose.

2.5.4.3 *International Trade*

An additional aspect of risk management is the requirement for compliance with regulations of animal-derived foods in international trade. Because there is no harmonized worldwide legislation on residues, barriers to international trade can and do arise through distortion of the conditions of competition in the market. Imported foods must comply with either CAC or national MRLs of the importing country. When MRLs have been established, the imported food can be subjected to a testing program. However, in some instances the MRL of the importing country may be lower than that of the exporting country. Moreover, no MRLs may have been established and then a zero-tolerance approach to residues is normally adopted. These varying circumstances can be addressed in several ways: through trade agreements between trading partners, by establishing import MRLs, and through assignment of export slaughter intervals (ESIs) to veterinary products intended for use in food-producing animals destined for overseas markets. The latter approach is unique, at present, to the Australian authority (APVMA). The ESI is defined as the interval between product administration and slaughter for export. It is determined first by a consideration of trade data for meat and edible offal and the degree of risk acceptable to major stakeholders. Then, a suite of algorithms is used to calculate

the probability of lot rejection of meat consignments, when treated animals are killed at various intervals. The ESI is that time when the upper tolerance limit about the regression line for the censored data intersects with the residue concentration associated with the acceptable risk. If a MRL has not been established by the importing company, the ESI endpoint is taken as the LLOQ of the analytical method.[105]

2.5.5 Risk Communication

The aims in risk communication are first to involve or inform all participants in the food chain, including marketing companies, regulatory authorities, and consumers, of the nature of risks associated with drug residues in foods, and then to provide assurance that precautionary principles have been applied to the generation and interpretation of data and the adoption of standards that ensure a safe food supply.

In large part, the risk communication and management procedures encompass the link between prescribing veterinarian and farmer. Farm animal veterinarians, at least in the EU, have more recently assumed a significantly increased role as educators of their clients in the safe and effective use of drugs and the maintenance of adequate records. The latter should constitute an essential element in ensuring compliance with effective therapy and adherence with statutory WhTs. Quality assurance programs, instituted by veterinarians and producers, have made a significant contribution to reducing the occurrence of non-compliant residues and thereby providing assurances on food safety to the public.[125,126]

2.6 RESIDUE VIOLATIONS: THEIR SIGNIFICANCE AND PREVENTION

2.6.1 Roles of Regulatory and Non-regulatory Bodies

Residue violations may occur as a consequence of the use of drugs and pesticides or from environmental contaminants and naturally occurring toxicants in foods. Drugs (including pesticides registered for veterinary use) are the most commonly detected chemicals in animal-derived foods and, of these, a large majority of positive findings are AMDs. Dowling has outlined the roles of the US Department of Agriculture's Food Safety and Inspection Service (FSIS) and the Canadian Food Inspection Agency (CFIA) in monitoring meat, poultry, eggs, and honey for residues of chemicals, including AMDs.[51] FSIS monitors tissues through its National Residue Program (NRP). Both agencies utilize hazard analysis and critical control point (HACCP)-based systems as the basis for conducting risk analyses.

Annually, FSIS and CFIA analyze approximately 300,000 and 200,000 samples, respectively, from all market classes of food-producing animals. When a non-compliant residue is detected in a slaughter animal or food animal product, it is condemned. FSIS informs USFDA of residues violations and seeks to obtain the names of producers of products and/or to identify other parties offering animals or products for sale. Appropriate action by the federal agency may include follow-up inspections, seizure and recall of products, and, on the basis of a surveillance plan, further sampling. The action taken depends on the magnitude of the health risk, and emphasis is placed on avoidance of any repeat occurrence and/or further distribution of products.

The standard adopted by FSIS and CFIA is that the incidence of non-compliant residues should not exceed 1%, when veterinary drugs are administered according to label instructions. Any value greater than 1% is deemed to indicate that a product has not been used in accordance with label directions. As discussed by Paige et al., examples include administration to a non-approved species, administration of doses exceeding the recommended maximum, administration by a non-approved route, failure to adhere to prescribed WhTs, failure to maintain treatment records (and thereby failure to identify treated animals), and administration of drug products in error.[127]

Another cause of non-compliant residues, notably in culled dairy cows and veal calves, is the salvaging of animals for slaughter following AMD treatment. Dowling reported that the consumption of medicated feeds is a common cause of residue violations in pigs and poultry, partly as a consequence of the difficulties involved in adhering to WhTs.[51] Adherence may be both expensive and inconvenient, in that it involves replacement of medicated with non-medicated feed in the WhT. Contamination of compound feeds can also arise through inadequate processes in mills, including plant design, and inadequate pre-mix formulation. Croubels et al. list problem compounds as sulfonamides, tetracyclines, nitroimidazoles, nitrofurans, nicarbazin, and ionophore coccidiostats.[35] In some jurisdictions, products can be used in a non-approved species, under the responsibility of the prescribing veterinarian and based on estimation of a suitable WhT. Such recommendations may be based on estimations lacking sufficient accuracy. Gehring et al. have discussed the application of risk management principles to the extra-label (off-label) use of drugs.[128]

As discussed below, various residue detection programs are in use. All are designed to minimize the incidence of non-compliant residues. In the matter of prevention, the role of the Food Animal Residue Avoidance Databank (FARAD) should be noted. FARAD is a USDA-supported computerized databank, established in 1982, with the objective of minimizing residue violations, through the collection, collation, and dissemination of information relevant

to residues prediction. It is a cooperative project between North Carolina State University, the University of Florida, and the USDA. FARAD collates information on approved animal drugs, extralabel drug use and environmental toxins on a searchable computer database. It incorporates data in an on-line database (VetGRAM) for more than 1000 drugs and chemicals and more than 20,000 published pharmacokinetic studies. The latter includes a range of pharmacokinetic parameters and variables (clearance, volume of distribution, half-life, maximum concentration, etc.) on drugs, pesticides, and environmental contaminants with potential for presence in livestock tissues. Mathematical models of residue depletion have been developed from the pharmacokinetic data; these predict residue depletion profiles, irrespective of administered dose. A second role of FARAD, both educational and consultative, is to provide advice on residue avoidance and mitigation for chemical contamination incidents and the extralabel use of drugs. The database also provides regulatory information on

1. Indications and directions for use of drugs in food-producing animals for therapeutic and production-enhancing purposes
2. Toxicokinetic data
3. Foreign registration and safety data
4. Tolerances of AMDs in tissues, eggs, and milk
5. Withholding times
6. Bibliographic citations

All FARAD pharmacokinetic data have been published in book form.[109] It is regularly updated. Of 912 enquiries to FARAD reported in 2003, the greatest numbers were for AMDs (338) and NSAIDs (143). Of AMD enquiries the greatest numbers related to dairy cattle, followed by pigs and beef cattle.[129]

In 1998 the concept was extended to development of a global(g) FARAD, embracing several countries and facilitating international dissemination of data on drugs used in food animals and residue avoidance. The collaboration extends to sharing data on withdrawal recommendations, interspecies extrapolations, and the extralabel use of drugs. Data may be obtained from the FARAD organizations at FARAD@ncsu.edu, FARAD@ucdavis.edu, or www.farad.org for the United States and cgfarad@umontreal.ca or www.cgforad.usask.ca for Canada. The FARAD advisory service has particular value in relation to the extralabel use of AMDs, for example, use of a dose different from that authorized or in a different food-producing species. This was legalized in the United States with passage of the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA).[130] AMDUCA applies solely to FDA-approved animal and human drugs administered only by or under the order of a licensed veterinarian.[131] AMDUCA does not permit extralabel use of drugs in feed and also specifically prohibits the extralabel use of fluoroquinolones, glycopeptides, furazolidone, nitrofurazone, chloramphenicol, dimetridazole, ipronidazole, other nitroimidazoles (such as metronidazole), and sulfonamide drugs in lactating dairy animals (except for approved uses of sulfadimethoxine, sulfabromomethazine and sulfaethoxypyridazine). Under AMDUCA the hierarchy of use of AMDs in food-producing animals is

1. A product approved for the condition being treated that is effective as labeled
2. A product approved for a food animal that may be used in an extralabel manner
3. A product approved in non-food-producing animals or humans that may be used in an extra-label manner

If no products exist that satisfy these requirements, a compounded product may be permitted.

In the EU, the 1999 ban on the use of AMDs as growth promoters included avoparcin, ardacin, zinc bacitracin, spiramycin, tylosin, virginamycin, carbadox, and olaquindox. Also in the EU, avilamycin, flavomycin, salinomycin, and monensin were phased out in 2006.

In addition to the several classes of residue detection program and the excellent FARAD program, there are non-regulatory approaches to minimizing the incidence of non-compliant residues. Professional associations (e.g., the UK National Office of Animal Health) and academics training future veterinarians have roles to play in drawing attention to guidelines issued by the local jurisdiction. Roeber et al. stress the importance of the responsible use of drugs on farms and the implementation of quality assurance programs by producers.[126] There is a key role for veterinarians through the provision of advice to farmers on matters of good practice and injection technique to reduce wastage of meat as a result of trimming blemishes. Pharmaceutical companies will continue to make advances in product formulation and drug delivery technologies. As potential means of tackling injection site residue problems, the use of biodegradable polymers as drug carriers, injectable microspheres, and microcapsules may be mentioned. For sustained drug release at intramuscular injection sites, the use of liposomes as carriers has been reported. Finally, there is a range of residue detection programs now in use, and their specificity and sensitive increases with time.

2.6.2 Residue Detection Programs

Federal and national agencies have adopted residue detection (control) programs for both domestically produced and imported products. The control programs vary, as they are structured according to the needs of the particular country, but overall it might be noted that published results

from these programs provide overwhelming evidence of a safe food supply. The methods used are of two classes, screening and confirmatory. The former detect the presence of an analyte and have the capacity for high sample throughout, checking large sample numbers for potential non-compliance, that is, positive results. The latter enable the analyte to be identified unequivocally and if necessary quantified at the concentration of interest (e.g., at 0.5 MRL). They utilize a range of methods, with many laboratories moving progressively to mass spectrophotometric methods, as described in subsequent chapters of this text. Additionally, domestic residue sampling is classified into four categories according to purpose: monitoring, enforcement, surveillance, and exploratory.[51] Another means of classifying methods is on the basis of analytical principle used, namely, physicochemical, immunochemical, and microbiological. Immunochemical assays are further subdivided into immunoassays, including enzyme-linked immunosorbent assay (ELISA) and immunoaffinity chromatography (IAC). Physicochemical methods are based on chromatographic purification following spectroscopic quantification such as ultraviolet, fluorescence, or mass spectrometric methods. Microbiological methods comprise rapid screening tests. They are inexpensive, provided an extraction procedure is not required. Domestic programs are a trade requirement, either mandatory or as an expectation of importing countries. For detailed discussion, see Croubels et al.[35] and De Brabander et al.[132]

2.6.2.1 Monitoring Program

These programs involve a statistically based selection of random samples, each analyzed for selected drugs, collected from healthy animals at slaughter. Animals or animal-derived foods (eggs, milk, honey) sampled are normally those that have passed ante- and postmortem inspections. The residues are assessed for compliance with the MRL. Food products are not retained prior to analysis, but may be recalled, if analyses suggest a public health concern. The data obtained are used to evaluate trends, such as drug misuse, which may lead subsequently to a targeted sampling program. The number of animals sampled is selected to provide a 95% probability of detecting one or more violations when 1% of the animal population contains a non-compliant residue concentration, that is, exceeding the MRL.[133]

Dowling summarized data obtained from United States (FSIS) and Canadian (CFIA) monitoring programs (predominantly meat, eggs, and honey) in 2003.[51] For example, FSIS identified 87 residue violations from 26,214 samples. Of these, 5608 samples analyzed for antimicrobial residues (excluding sulfonamides) yielded 36 violations. Most non-compliant residues related to neomycin, for which there were 29 violations in veal calves. In addition, there were 14 violations for sulfonamides from 5276 samples. Dowling also reported data for CFIA for 2002/03.[51] It is of interest to note for CFIA analyses, using microbial inhibition tests, the presence of inhibitors in 42 of 312 rabbit samples, while of 1055 honey samples, non-compliant residues were reported for erythromycin (2), oxytetracycline (14), sulfathiazole (2), and tetracycline (10).

In the EU, monitoring programs are conducted according to Council Directive 96/23/EC;[134] residue substances and the number of samples to be tested are defined in Annex I of Directive 96/23.[45] Included are all AMDs with MRLs (group B), including those incorporated in feedstuffs, and banned compounds listed in Annex IV of Council Regulation 2377/90 (group A).[44] The AMDs in group A are chloramphenicol, dimetridazole, metronidazole, ronidazole, and nitrofurans, including furazolidone. For banned substances in the EU, emphasis is placed on identification in a large number of matrices, including meat, urine, and even hair at a concentration as low as possible, in accordance with the principle of zero tolerance. Accordingly, the minimum required performance limit (MRPL) is set at a concentration 10–100 times lower than those generally used for MRLs. For banned substances the requirement is for a positive finding from an initial qualitative multi-residue method to be followed by confirmation of identity using a method that provides sufficient identification points, as specified in EU Commission Decision 2002/657/EC.[45]

As an aside, one might mention hair as a generally very stable matrix for most drugs and metabolites.[135] These authors detected quantitatively sulfonamide and trimethoprim in horse tail hair 3 years after oral dosing in a horse, at a distance of 45–55 cm from the follicle. For the nitrofurans a MRPL of 1 μg/kg has been set in the EU. "Bound Residues and Nitrofuran Detection" (FoodBRAND) comprises a rapid multi-residue screening test and also includes definitive multi-residue reference methods for protein-bound remnants of the nitrofurans.[132]

In addition to the tests conducted by regulatory authorities, major abattoirs often have their own quality assurance programs to ensure freedom of products from non-compliant residues, particularly in products destined for export markets, which may impose a different regulatory limit for certain residues than their national authority. Results of such tests are seldom included in totals published by national authorities, unless their inclusion is clearly stated.

2.6.2.2 Enforcement Programs

The object of these programs is to analyze samples from animals judged to be at high risk of having non-compliant residues. High-risk expectation may be due to historical data for a product group and includes appearance on ante- and post-mortem inspections. In North America, typically suspect animals include young calves (aged <3 weeks and weighing <68 kg), culled dairy cows, a visible injection site, animals of a production class in which a residue

monitoring program has revealed a high incidence of non-compliant residues, and animals in which there is evidence of infectious disease.

Initial "in plant" tests using microbiologically based rapid screening methods of detection for AMDs, include the "Swab Test on Premises" (STOP), "Fast Antimicrobial Screen Test" (FAST), "Overnight Rapid Beef Identification Test" (ORBIT), "Calf Antibiotic and Sulfonamide Test" (CAST) and "Sulfa-on-Site" (SOS). Also used are a range of ELISAs. Carcasses from suspect animals are retained at abattoirs pending the results of tests. If the screening test gives a positive result, FSIS or CFIA carry out a confirmation test. In the absence of availability of a screening test or if a residue not detected by FAST or STOP is nevertheless suspected, tissue samples are submitted directly to FSIS or CFIA. If the confirmatory test is positive for a non-compliant residue, the carcass is classified as adulterated and condemned. Tests for use "on site" are also available for detecting AMDs in honey, such as the CAP Residue Rapid Inspection Device for chloramphenicol and the Tetrasensor Honey test, which detects four tetracyclines. Novel electrochemical and optical immunosensors, flow cytometric immunoassays, and biochip assay methods for residue analysis are currently under development.[132] Rapid tests are discussed further in Chapter 5.

Data from enforcement programs are evaluated to ascertain their effectiveness in reducing residues. In 2003, FSIS recorded 1923 residue violations from 230,351 samples; these violations included 1470 for AMDs (excluding sulfonamides), 335 for sulfonamides, and 118 for the non-steroidal anti-inflammatory drug, flunixin. A full analysis is provided by Dowling.[51] Of interest because of high incidence in the FAST test on 215,813 samples were 552, 199, and 195 violations for penicillin, sulfadimethoxine, and gentamicin, respectively, all in dairy cows and 372 neomycin positives in young veal calves. These represent high percentages of the 1820 (0.84%) total non-compliances in 1665 animals. The STOP test on a total of 14,360 samples revealed 28 positives for penicillin in dairy cows. These represent a high proportion of the 74 (0.51%) total non-compliances in 64 animals. Similar data generated by CFIA on suspect animals in 2003 in SOS, STOP, and CAST tests, with confirmatory tests, indicated the highest incidence of non-compliances for oxytetracycline (120) and benzylpenicillin (182) from a total of 346 non-compliant samples from 11,877 samples analyzed. The great majority of samples related to pork and beef. In both Canada and the United States, food producers and distributors who violate standards are placed on enhanced inspection, with the objectives of identifying causes and reducing non-compliances.

2.6.2.3 Surveillance Programs

The objective of surveillance sampling is to evaluate the occurrence and incidence of a potential residue concern in a particular animal population, when there is suspicion of non-compliant residues on the basis of herd history or clinical signs. The data obtained provide regulatory authorities with information on whether residues have been reduced by interventions. Carcasses or products may or may not be retained pending laboratory findings; this depends on the nature and weight of the evidence that initiated the surveillance and also varies according to the policies of the particular jurisprudence. Normally, the source of supply is traced and action is taken to ensure non-recurrence. The action may include seizure and disposal of produce, quarantining a farm, additional residue testing at the expense of the producer, and prevention of sale of produce until the commodity has been shown to be safe for consumption and acceptable for sale in domestic and export markets. Additional potential procedures include implementing industry codes of practice, auditing users and operators, and obtaining feedback from sellers. Dowling quotes the example of submitting 6295 market hogs for FSIS screening for sulfonamides; the SOS test revealed 10 sulfamethazine non-compliances.[51]

In the UK, the Veterinary Medicines Directorate has monitoring programs, the results of which are published periodically in the *Veterinary Record* and in the annual report of the Veterinary Residues Committee.[137]

2.6.2.4 Exploratory Programs

Exploratory residue testing is conducted on drugs that do not have MRLs. The objective is to evaluate new methods and approaches for both monitoring and sampling. Exploratory testing generates information on non-MRL drugs, but is not designed to facilitate regulatory action.

2.6.2.5 Imported Food Animal Products

In addition to residue monitoring, enforcement, surveillance, and exploratory programs, designed to evaluate residues in animal-derived foods produced in the country of origin, regulatory authorities conduct inspections on imported foods. These are actually re-inspections, the extent of which depends on previous knowledge of the particular exporting country's standards. Each importing country seeks to verify that imported foods meet the same standards as those operating under the domestic programs. As discussed by Dowling, in 2003 the United States tested various foods (processed meat, poultry, and eggs) for residues in eight compound classes of veterinary drugs and pesticides.[51] Of 2212 samples tested, two residue non-compliances were identified, both for the anthelmintic, avermectin. Interestingly, in 2003 Canadian import testing revealed 27 and 6 detections of chloramphenicol in honey imported from India and the United States, respectively.

2.6.2.6 Residue Testing in Milk

The problems and penalties associated with residues of AMDs in milk and other dairy products have given rise

to several tests for monitoring non-compliant residues of AMDs in milk. These include receptor-binding assays, microbial receptor assays, microbial growth inhibition assays, enzyme assays, and chromatographic analyses. These are used to test bulk tank milk and include the following: Charm SL-Beta-lactam, DelvoTest P5 Pack Beta-lactam, IDEXX SNAP Beta-lactam, and Charm II Tablet Competitive Beta-lactam. The Delvo test is very widely used. More recently, rapid tests, providing results in 3 min, have been described, for example, Charm MRL-3 and β-STAR 1+1 (STAR = Screening Test for Antimicrobial Residues). The Parallux Milk Residue Testing System detects six β-lactams, tetracyclines, spectinomycin, neomycin, streptomycin, spiramycin, sulfonamides, and quinolones in one test within 4 min.[132] As these commercial names indicate, there is particular concern over the presence of non-compliant concentrations of AMDs of the β-lactam group (penicillins and cephalosporins). In fact, it is rare for inhibitor-positive milk to contain antibiotics other than β-lactams. Most methods therefore used *Geobacillus stearothermophilus*, which is very sensitive to β-lactams (see Chapter 5. for further discussion). Riviere and Sundlof point out that summarizing available tests is difficult because of rapid advances in methods leading to new tests.[123]

In 2003 in the United States, 4,456,141 tests for residues in milk and other dairy products yielded 3246 (0.07%) positive results. Of these, 4,354,087 tests and 3207 positives (0.07%) were for β-lactam drugs.[138] Of 66,124 tests for sulfonamides, 23 (0.03%) were positive. In the same year, of 3577 milk and cheese products tested by CFIA, none yielded positive results for AMDs or sulfonamides. The screening tests in use have good sensitivity and negative predictive values, but poor positive predictive values. Thus, a positive test on an individual cow does not necessarily indicate a bulk tank milk concentration exceeding the MRL (see Chapter 5 for further discussion).

In setting withholding periods for AMDs in milk, regulatory authorities allow the assumption that milk from a treated cow will be admixed with milk from untreated animals; that is, MRLs for milk are based on bulk tank concentrations, prompting the comment that "the solution to pollution is dilution." This is not unreasonable, as the human consumption of milk from a single animal will be a relatively rare event, at least in developed countries. An increased somatic cell count (SCC) of bulk milk is an indicator of the prevalence of mastitis in a dairy herd. Such infections are widespread and are therefore widely and routinely treated by AMDs administered by intramammary infusion (most countries) or systemically (Scandinavian countries). Cases of peracute mastitis will almost always be treated systemically in all countries. There is an expectation that a non-compliant residue is more likely to occur in milk from herds with a high SCC. The US Pasteurized Milk Ordinance requires all bulk milk tankers to be sampled and analyzed for AMD residues prior to processing.[139] Additionally, at least four samples from pasteurized milk and milk products are required to be tested from each plant at 6-month intervals, and each producer must be tested at least 4 times every 6 months. Typically, dairies include additional testing for their own purposes to ensure the freedom of milk from residues that pose a risk to the consumer, or to manufacture of products such as yogurt and cheese. Results of these tests are seldom included in the totals published by national authorities.

2.7 FURTHER CONSIDERATIONS

2.7.1 Injection Site Residues and Flip-Flop Pharmacokinetics

When drug products are administered to animals by a parenteral (other than the intravenous or oral) route, usually intramuscularly or subcutaneously, drug concentrations at the injection site are initially always high. As drug is absorbed into the circulation, the concentration falls rapidly. In the case of drugs administered as aqueous solutions, such as aminoglycosides and the sodium or potassium salt of a penicillin, the drug normally remains in solution at the injection site and complete absorption is generally very rapid. Thus, for sodium benzylpenicillin administered intramuscularly, T_{max} occurs within 10–20 min of administration. In this circumstance, depletion from the injection site is sufficiently rapid to ensure that the concentration in injection site muscle decreases to a concentration not distinguishable from non-injection-site muscle by the time of slaughter. Therefore, marker tissue will not be injection site muscle, and may or may not be non-injection-site muscle.

However, for many AMDs and indeed drugs of other classes (e.g., anthelmintics), there has long been a practice of developing slow-release (depot) formulations, administered intramuscularly, subcutaneously, or as "pour-on products," for use in farm animal species. As discussed in Section 2.2.3, these products commonly display flip-flop pharmacokinetics, in which the terminal half-life represents a slow absorption phase and is longer than the elimination half-life determined after intravenous dosing. The advantages and disadvantages of depot preparations are summarized in Table 2.15.

The potential complexity of the pharmacokinetic profile for slow-release products is illustrated by the early study of Toutain and Raynaud.[87] These workers administered a 20% w/v solution of oxytetracycline intramuscularly to young calves at a dose rate of 20 mg/kg. The data best fitted an open two-compartment model (central and peripheral) with two absorption compartments (rapid and slow release). The rapid absorption phase was attributed to immediate

TABLE 2.15 Advantages and Disadvantages of Parenteral Depot Formulations of AMDs

Advantages	Disadvantages
Provide products with long duration of action, requiring single dosing and/or or a long (48–72-h) dosing interval	Generally have much longer withholding periods than rapidly absorbed formulations, discouraging innovative developments and field use
Greater convenience and lower cost than products requiring more frequent dosing (e.g., once daily for 3–4 days)	Burden for farmers required to adhere to prolonged withholding period
Greater compliance with administration of dosage schedule	Greater risk for consumers through possible non-adherence to prolonged withholding periods
Improved consumer safety through greater compliance	Possibly pain and inflammation at injection sites, leading to a local response involving formation of fibrous granulation tissue that can enclose and create a "protected pocket" containing AMD
Improved animal welfare by minimizing stress of handling and pain on repeated injection	Problems for regulatory authorities in setting withholding periods based on slow depletion from injection sites
	Threats to international trade through persistence of residue at injection sites

availability of a small fraction (14%) of the administered dose, with an absorption half-life of 48 min. The slow absorption phase was associated with a larger fraction (37.5%) of the administered dose, with a half-life of 18.1 h. As elimination half-life after intravenous dosing was 9.04 h, the product displayed flip-flop pharmacokinetics.

Some depot products have given rise to injection site residue concerns, as discussed in official guidelines (e.g., see Ref. 140), in a review in 2007,[141] and in peer-reviewed articles.[142,143] The quantity of administered drug is generally large in sustained-release products, as it is required to provide both initially high and then well-maintained therapeutic concentrations (over $\geq$2 days). Injection site residues of slow-release formulations are likely to be less of a problem when absorption is steady, but in practice depletion is often erratic and non-exponential and therefore unpredictable. This seems to arise partly because the products, to achieve prolonged release, are formulated as suspensions in either water (e.g., benzathine and procaine benzylpenicillins) or water-repelling fixed oils (e.g., procaine benzylpenicillin) or as solutions containing organic solvents [e.g., high-strength (20–30%) oxytetracycline]. For the latter, after intramuscular or subcutaneous dosing, the rapid absorption of organic solvents leads to precipitation of the AMD, which is then slowly taken up into solution in interstitial fluid at the injection site. In addition, variable amounts of the suspension or precipitate may induce a local acute inflammatory response and/or, as a foreign body, become walled off by granulation tissue and therefore subject to very slow and erratic absorption.[80,82]

There is a lack of consistency between jurisdictions in addressing the issue of injection site residues.[141] One reason for the lack of harmonization of risk assessment is the limited data available on the probability of dietary exposure to injection sites containing residues.[144] The paucity of data extends to three areas: (1) prevalence of injection site tissue at slaughter, (2) incidence of remaining residues above MRL at injection sites, and (3) the fate of injection sites. Determination of prevalence is confounded by regional differences in animal husbandry practices, so that extrapolation cannot be made from data obtained in one region to another. Injection site tissue is trimmed out and discarded when identified, and the efficiency of this procedure (although unknown) is inevitably variable. There are only limited data available on percentages of injection sites that actually contain residues, but the proportion may be small. However, Beechinor and colleagues *have* generated good data on (1) injection site muscle residues of tilmicosin, tiamulin, and enrofloxacin in livestock in Ireland[145] and (2) prevalence and public health significance of blemishes in cuts of Irish beef and pork.[146,147]

Several regulatory approaches have been taken to address injection site residues. The one adopted in some jurisdictions (e.g., USA and Australia) is to use ARfD in place of ADI as the permissible exposure standard. This is reasonable, but the validity of this approach depends on consumption of injection site residues being a rare event. Use of ARfD will shorten the WhT, provided its value exceeds that of ADI, and if muscle is the target tissue. An additional consideration is that this approach requires a residue surveillance sampling protocol that can differentiate between injection site and non-injection-site muscle. At several meetings of the CCRVDF, through the 1990s and 2000s, this was proposed but regrettably not adopted. Thus, in 2001 a working paper discussed by the CCRVDF proposed the analysis of two muscle samples; two positive results would indicate violation of the WhT, whereas a single positive would indicate an injection site sample, in which event the ARfD could be applied and violation would occur only if the positive value exceeded ARfD. EMA/CVMP envisaged three practical problems relating to this proposal: (1) injection sites

might not be easily identifiable, and moreover it was possible that only a part of an injection site would be sampled; (2) in some cases additional analytical method validation might be required; and (3) an additional analytical method might be needed if the marker residue at the injection site (normally a parent drug) differed from the marker residue in non-injection-site muscle. EMA/CVMP therefore continues to require injection site muscle to be treated as non-injection-site muscle; specifically, the former must decline below the MRL. This was the standard adopted when all benzathine benzylpenicillin–containing parenteral products were banned in the EU; the argument was that potentially serious allergenic consequences of consumption of small amounts (above the MRL of 50 μg/kg) could arise, even if consumption was a very rare event.

In the present authors' opinion, acceptable advances might be made in addressing injection site residue issues through consideration, development, and possible adoption of the proposals of Sanquer et al.[142,143] They questioned the EMA/CVMP guideline, which recommends application of the same calculation method for injection site muscle as other edible tissues. This was considered to be scientifically unsound, on the grounds that injection site residues often violate regression assumptions regarding both homoscedasticity (same variance in residue concentrations for different slaughter times) and linearity (of mean depletion curve in $\log_e$ scale). These authors applied a probabilistic approach in assessing risk of consumption of an injection site, in whole or in part, during one year, based on a 7-day AMD treatment. The analysis indicated, for EU consumers, a maximal risk of 4 days of injection site consumption (containing or not containing residues). They proposed a nonparametric approach for calculation of WhT, stating that acute risk exposure associated with injection site consumption could be more appropriately dealt with by use of ARfD or acute single-dose intake (ASDI) indices. In earlier studies, Concordet and Toutain had already proposed a nonparametric approach as an alternative to the recommended statistical approach.[111,112]

2.7.2 Bioequivalence and Residue Depletion Profiles

Many of the antimicrobial drug products licensed for use in food-producing species are generics, that is, products containing one or more drugs developed initially as pioneer products, but that have subsequently been formulated in products containing the same drug, usually (but not necessarily) in the same concentration and usually (but not necessarily) in a similar formulation. A crucial component of the data required by regulatory authorities for generic products is a study to determine whether the generic and pioneer products are bioequivalent. Bioequivalence allows applicants for MAs of AMDs, which are generic to a pioneer product, to claim essential similarity, in terms of efficacy and safety in each target species.

The assessment of bioequivalence is based on 90% confidence intervals for the ratio of the population geometric means (test/reference) for the parameters under consideration. This method is equivalent to two one-sided tests with the null hypothesis of bio-inequivalence at the 5% significance level. Two products are declared bioequivalent if upper and lower limits of the confidence interval of the mean (median) of log-transformed AUC and C_{max} each fall within the a priori bioequivalence intervals 0.80–1.25. It is then assumed that both rate (represented by C_{max}) and extent (represented by AUC) of absorption are essentially similar. C_{max} is less robust than AUC, as it is a single-point estimate. Moreover, C_{max} is determined by the elimination as well as the absorption rate (Table 2.1). Because the variability (inter- and intra-animal) of C_{max} is commonly greater than that of AUC, some authorities have allowed wider confidence intervals (e.g., 0.70–1.43) for log-transformed C_{max}, provided this is specified and justified in the study protocol.

Regulatory bodies in general accept that, although the pioneer and generic products are not pharmacokinetically identical, they are nevertheless deemed to be *sufficiently similar* to permit the assumption that they will be therapeutically equivalent. Therapeutic equivalence is taken to mean that the products will have the same efficacy and safety profiles in the target species. This, in turn, means that the company seeking to license the generic product will not normally have to undertake the otherwise extensive laboratory animal and target species safety studies and clinical trials to establish safety and efficacy in clinical use, assuming that the claims for the generic product are identical to those made for the pioneer product.

In relation to residues in food-producing species, it is important to recognize that demonstration of average bioequivalence does not obviate the need for separate residue depletion studies for a generic product. There are several reasons why this is so:

1. It might be noted the definitions of WhT and average bioequivalence are fundamentally different; it should be stressed that to guarantee a 90% confidence interval for the ratio of the two treatment means of AUC and C_{max}, respectively, it should be entirely contained within the range 80–125%. Meeting this criterion gives no guarantee that the upper 95% confidence limit of the 95th percentile of the population is below the MRL. Indeed, bioequivalence can be determined at three levels: average, population, and individual. It is outside the scope of this chapter to discuss each of these, except to say that average bioequivalence is the most easily satisfied (least stringent) of the three. Regulatory

authorities require companies to demonstrate average bioequivalence only, and two formulations can be declared bioequivalent while their variances for AUC are different, and this may have a large impact on WhT that controls a population percentage, not a mean parameter. Only a so-called population bioequivalence could also guarantee equivalence of variance.

2. It is clear that, for a parenteral product administered intramuscularly or subcutaneously, depletion from the injection site may well be sufficiently similar to provide bioequivalence variables that fall well within the preset limits, but are nevertheless sufficiently different to yield significant, even quite large, differences in concentration at the injection site.[141]
3. It is not only at the actual injection site, however, that residue depletion is likely to differ; depletion rate is not guaranteed either for *all* edible tissues. This is because bioequivalence demonstrates essential similarity in rate and extent of absorption between a pioneer product and a generic product only for the range of therapeutically useful plasma concentrations. Bioequivalence does not guarantee the same rate of decrease of concentration in the terminal phase, which often has no therapeutic meaning. For many drugs (see Section 2.3.1 for an example of gentamicin), a rapid elimination phase is followed by a much slower decline in concentration ($T_{1/2}$ values of 1.83 and 44.9 h, respectively, for β and γ phases for gentamicin in calves). The γ phase represents the unloading and elimination of drugs from tissues, including edible tissues. It is not possible for average bioequivalence established over the first 24 h following product administration to give assurance on the same exposure in the γ phase, that is, much later (in days or even weeks). In addition, a very late terminal phase may reflect a flip-flop phenomenon undetected at the plasma level by a conventional bioequivalence trial. **For all these reasons, there is no (statistical) basis for having the same WhT for different generic products and a pioneer product.**

2.7.3 Sales and Usage Data

Sales and usage of AMDs inevitably vary considerably between and even in regions within countries. As examples, in this section we will consider recent sales data for two countries (United Kingdom and France) and sales data for a single clinical condition (bovine respiratory disease, BRD).

2.7.3.1 Sales of AMDs in the United Kingdom, 2003–2008

The United Kingdom's Veterinary Medicines Directorate Food and Rural Affairs) 2009 report describes sales of AMDs, antiprotozoals, antifungals, and coccidiostats, authorized for use as veterinary medicines, annually for the period 2003–2008.[148] To illustrate trends in usage, this account summarizes data for 2003 and 2008. Prior to that, between 1998 and 2003, the total sale of veterinary therapeutic AMDs in the UK was relatively constant at approximately 434 metric tons per annum. From 435 metric tons in 2003, total sales decreased to 384 metric tons in 2008. Livestock numbers in thousands in the national herd (2003 and 2008) were as follows: pigs (5046 and 4714), cattle (10,508 and 10,107), sheep (35,812 and 33,131), and poultry (178,800 and 166,200). Therefore, the proportional decreases in total AMD sales and in animal numbers were broadly similar.

Sales figures for AMDs and coccidiostats are presented in Table 2.16. The decrease in tonnage sales of AMDs in food animals is partly accounted for by the ban on growth promoters, which took effect on January 1, 2006. For AMDs, it will be seen that by far the largest tonnage (59% of total) relates to medicated feedstuffs, followed by products formulated for oral or water medication (29%) and injectable medication (10%). For intramammary products, 56.6% was for dry-cow and 43.4% for lactating cow therapy. Of the coccidiostats, 72% comprised ionophores. It should be noted that the proportion of the 327 metric tons of AMDs, which was administered to food animals but did not enter the food chain, is unknown.

Categorized by species, by far the largest groups of AMD sales were for pigs and poultry, with smaller and

TABLE 2.16 Sales of Therapeutic AMDs and Coccidiostats in UK in 2003 and 2008 (Metric Tons of Active Ingredient)

Category	2003	2008
Therapeutic AMDs		
Total sales	435	384
Food-producing animals only	377	327
Combination of food and non-food-producing animals	28	18
Non-food-producing animals only	30	38
Growth-promoting products	36	0
Medicated feedings stuffs	307	228
Oral/water medication	87	112
Injectables	34	38
Intramammaries	5	4
Intramammaries[a]	4735	4092
Dry cow[a]	2590	2317
Lactating cow[a]	2145	1775
Coccidiostats		
Total coccidiostats	240	207
Ionophores	190	150
Non-ionophores	50	57

[a] Values in kilograms, not metric tons.

TABLE 2.17 Sales of Therapeutic AMDs in UK in 2003 and 2008 (Metric Tons of Active Ingredient) by Species and Chemical Groups

Product	2003	2008
Species		
Cattle only	12	11
Pig only	70	62
Poultry only	11	31
Fish only	2	1
Pig plus poultry	261	195
Multiple edible animal species	21	28
Chemical group		
Tetracyclines	212	174
Trimethoprims/sulfonamide	89	70
Trimethoprims	15	12
Sulfonamides	74	58
β-Lactams	62	69
Cephalosporins[a]	3(3037)	6(6242)
Penicillins[b]	16	13
Other penicillins[c]	43	50
Aminoglycosides	21	18
Streptomycin	7	6
Neomycin + framycetin	5	1
Other aminoglycosides[d]	9	11
Macrolides	39	35
Fluoroquinolones[a]	1(1364)	2(1928)
Others	12	15

[a]Values in kilograms given in parentheses.
[b]Includes potassium and procaine salts of benzylpenicillin.
[c]Includes cloxacillin, amoxicillin, ampicillin, nafcillin, and penethamate hydriodide.
[d]Includes gentamicin, apramycin, kanamycin, and spectinomycin.
Source: Data from Veterinary Medicines Directorate (2009).[148]

much smaller tonnages used in cattle and fish, respectively (Table 2.17). Of the 195 metric tons of AMDs in the pig–poultry category, VMD has estimated 60% usage in pigs, 38% in poultry, and 2% were sold off-label for use in other (non-authorized) bird species (e.g., duck, turkey, game). Classified by chemical grouping, by far the largest category is the tetracycline group (45% of total sales) followed by potentiated sulfonamides and β-lactams (18% each). Most tetracyclines were sold for pigs and poultry as medicated feedstuffs under veterinary prescription. While total tonnages were small, it is of interest to note increasing trends in the sales of fluoroquinolones and cephalosporins and the decreased use, between 2003 and 2008, of neomycin and framycetin, due to the withdrawal of neomycin from the market. In 2008, the total number of AMD, antiprotozoal and coccidiostat products sold (for all species, including non-food-producing animals) was 370, made up as follows: β-lactams 131, tetracyclines 46, trimethoprim/sulfonamides 41, others 41, aminoglycosides 28, fluoroquinolones 25, macrolides 22, coccidiostats 11, and antiprotozoals 10. AMD products can be imported into the UK, when no authorized products are available; sales of imported AMDs increased markedly from 159 to 3883 kg of active ingredient, between 2003 and 2008, but remained a very small proportion of total sales.

Because of the nature of the sales data harvested, it is not possible to specify, on an interspecies basis, the precise usage/sales data for particular drug classes. The VMD report emphasizes that there is no central record of the use of AMDs in animals in the UK. However, VMD has estimated, from liveweight slaughter data for cattle, pigs, sheep, poultry, and fish (5,327,000 metric tons in 2003 and 5,516,000 metric tons in 2008) that one metric ton of AMD was used to produce 12,898 metric tons of liveweight animal in 2003 and 16,869 metric tons of liveweight animal in 2008. These data correspond to the sale of 80 g (2003) and 60 g (2008) of AMD for each metric ton of liveweight animal slaughtered.

2.7.3.2 Comparison of AMD Usage in Human and Veterinary Medicine in France, 1999–2005

Moulin et al. compared tonnages of AMDs sold in human and veterinary medicine in France for the 7-year period from 1999 to 2005.[149] Data were compiled from the registers of the French Agency for Veterinary Medicinal Products (AFSSA ANMV) for animals and the French Health Products Safety Agency (AFSSAPS) for humans. Data in tonnages of active ingredients were related to animal and human biomasses to compare usages expressed in mg/kg of body weight (Table 2.18). While approximately 60% and 40% of total tonnages were in animals and humans, respectively, in relation to unit biomass, usage was 2.4 times higher in humans than in animals.

The highest sales in humans and animals were β-lactams and tetracyclines, respectively. Tetracyclines alone, in veterinary medicine, represented approximately 50.4% of all sales, while tetracyclines, sulfonamides/trimethoprim, β-lactams, and aminoglycosides combined accounted for more than 80% of AMDs used. During the 7-year period, sales of cephalosporins and fluoroquinolones in veterinary medicine increased by 38.4% and 31.6%, respectively. Nevertheless, as percentages of total veterinary sales, their use remained relatively small: cephalosporins 0.64% and fluoroquinolones 0.33% in 2005. In animals, oral administration accounted for 88% of sales, and estimated sales for parenteral products were 10.5%. Moreover, while 92% of total tonnage was intended for food-producing animals, 64% of cephalosporins were intended for pets.

The human/animal comparison revealed that some classes were used almost exclusively either in animals (aminoglycosides, amphenicols, polymyxins, tetracyclines) or in humans (nitrofurans). Expressed as percentages of total tonnage sales within each sector (animal or human), several differences were revealed as follows (animal first, humans second): tetracyclines 50.4 and 1.7,

TABLE 2.18 AMD Consumption and Biomasses Estimated in Humans and Animals in France from 1999 to 2005

	AMD Sales (metric tons)		Population Body Mass (metric tons)		AMD Sales Relative to Biomass (mg/kg live weight)	
Year	Human	Animal	Human	Animal	Human	Animal
1999	896.20	1316.31	3,597,843	17,122,220	249.1	76.9
2002	809.44	1331.53	3,709,154	17,268,049	218.2	77.1
2005	759.67	1320.10	3,810,215	15,795,105	199.4	83.6

Source: Data from Moulin et al (2008).[149]

TABLE 2.19 Global Animal Health Sales by Product Category, Species, and Country in 2005 in $ ($billions)

Product Category	Sales	% of Total	Species	Sales	% of Total	Country	Sales	% of Total
Antiparasitics	4.875	28	Dogs + cats	5.75	33	USA	6.29	36.1
			Cattle	4.885	28	China	1.095	6.3
Biologicals	3.655	21	Pigs	2.94	17	France	1.04	6.0
Antimicrobials	2.785	16	Poultry	1.94	11	Brazil	0.909	5.2
Medicated feed additives	1.915	11	Horses	1.045	6	UK	0.825	4.7
Other pharmaceuticals	4.180	24	Other food animals + aquaculture	0.85	5	Japan	0.793	4.6
						Germany	0.74	4.3
						Others	5.718	32.8
Total	17.41	100	Total	17.41	100	Total	17.41	100

Source: Data provided by A. R. Peters.[150]

TABLE 2.20 Estimated Sales of AMDs for BRD Therapy in Four Territories

Territory	Sales of AMDs ($millions)	Percent of Global BRD Market (%)
USA	250	36.1
EU	186	26.7
China	44	6.3
UK	32.5	4.7

Source: Data provided by A. R. Peters.[150]

TABLE 2.21 Analysis of US Market Share of Main Actives for BRD Therapy

Active	Product	Market Share	Withholding Period (days)
Tulathromycin	Draxxin	35	49
Enrofloxacin	Baytril	20	10–14
Ceftiofur	Excede		13
	Exenel	20	8
Tilmicosin	Micotil	18	60
Florfenicol	Nuflor	3.0	30–44
Ceftiofur	Naxcel	2.5	71
Danofloxacin	Advocin 180	1.5	8

Source: Data provided by A. R. Peters.[150]

sulfonamides/trimethoprim 18.8 and 2.9, aminoglycosides 5.9 and 0.2, polymyxins 4.9 and 0.2, β-lactams 8.3 and 51.6, and macrolides 9.0 and 14.8.

2.7.3.3 Global Animal Health Sales and Sales of AMDs for Bovine Respiratory Disease

Data for AMD usage and sales in the treatment of bovine respiratory disease (BRD) have been supplied by Professor A. R. Peters.[150] BRD is a major cause of reduced productivity and economic loss globally;[151] in the United States alone the annual total cost to the cattle industry is estimated to approach $2 billion. In 2005 the total global animal health market was estimated at $17.4 billion. Table 2.19 classifies this on the basis of product category, animal species, and country. Together AMDs and medicated feed additives constitute 27% of the total; cattle, pigs, and poultry together make up 56% of the total; and the United sales by country in $million were United States 1280, China 521, France 315, Spain 252, Germany 214, and UK 164.

Tables 2.20 and 2.21 present estimated data for sales of AMDs for treatment and prevention of BRD in three countries and the EU, together with an analysis of major products used in the United States. The market is dominated by the United States and EU, which together account for 63% of the global BRD market. The US market is dominated by two products containing macrolides (53% of total), three products containing ceftiofur (22.5% of total), and two products containing fluoroquinolones (21.5% of total). While florfenicol has a much smaller percentage

size of $250 million; 3% of this market is $7.5 million annually.

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CHEMICAL ANALYSIS OF ANTIBIOTIC RESIDUES IN FOOD

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A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data:
Chemical analysis of antibiotic residues in food / edited by Jian Wang, James D. MacNeil, Jack F. Kay.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-470-49042-6 (cloth)
1. Veterinary drug residues–Analysis. 2. Antibiotic residues–Analysis. 3. Food of animal origin–Safety measures. I. Wang, Jian, 1969– II. MacNeil, James D. III. Kay, Jack F.
[DNLM: 1. Anti-Bacterial Agents–analysis. 2. Chemistry Techniques, Analytical–methods. 3. Drug Residues. 4. Food Safety. QV 350]
RA1270.V47C44 2011
615.9′54–dc22
2010054065

Printed in the United States of America

ePDF ISBN: 978-1-118-06718-5
oBook ISBN: 978-1-118-06720-8
ePub ISBN: 978-1-118-06719-2

10 9 8 7 6 5 4 3 2 1